1

Vertebrates Originate

Key Questions in this Chapter

1) What are the closest living relatives of vertebrates? Did vertebrates originate as worms or bag-like beasts?
2) When did deuterostomes and chordates originate?
3) What are the key characteristics of chordates?
4) How do embryology and morphology, combined with new phylogenomic studies, inform us about the evolution of animals and the origin of vertebrates?
5) How do extraordinary new fossil discoveries from China help us understand the ancestry of vertebrates?

Introduction

Did vertebrates begin as worms or bags? This is the question. There are currently two viable hypotheses for the origin of vertebrates, called more properly the vermiform and the sessile hypotheses (Nanglu et al. 2023a). The vermiform hypothesis is that the ancestral vertebrate was like an enteropneust hemichordate, with a simple nervous system and a gill-slit-laden pharynx. The sessile origins hypothesis is that the ancestral vertebrate was a tentaculate animal that lived in a fixed position on the seabed and was perhaps more like a pterobranch hemichordate or a urochordate. We will look at what all this means, examining these close relatives of vertebrates as well as some spectacular fossils, and then summarize between worms and bags at the end of the chapter.

Vertebrates are the animals with backbones, fish, amphibians, reptiles, birds, and mammals. We have always been especially interested in vertebrates because this is the animal group that includes humans. The efforts of generations of vertebrate palaeontologists have been repaid by the discovery of countless spectacular fossils: heavily armoured fishes of the Ordovician and Devonian, seven- and eight-toed Devonian tetrapods, sail-backed synapsids, early birds and dinosaurs with feathers, giant rhinoceroses, rodents with horns, horse-eating flightless birds, and sabre-toothed cats. These fossils tell us where the living vertebrates have come from, and they show us glimpses of different worlds that are bizarre enough to defy the imagination of a science fiction writer. Despite all this information that has accumulated over the past 200 years, the origin of vertebrates is still hotly debated.

One thing is clear from the biology of living animals. Vertebrates are members of a larger group, the Phylum Chordata, which also includes their closest living relatives, marine animals such as the sea squirts and amphioxus (see below). These creatures do not have a skeleton, but they share other features, such as a **notochord**, a flexible, tough rod that runs the length of the body along the back. The notochord in living chordates is generally made from an outer sheath of collagen, a tough fibrous connective tissue that encloses turgid, fluid-filled spaces. Invertebrate chordates also have V-shaped muscle blocks (**myomeres**) along the length of their body. The question about the origin of vertebrates then broadens out to include the origin of chordates.

Looked at more widely, vertebrates are a minor twig among Animalia in the 'Tree of Life' (Figure 1.1). It is common to think of the major divisions of life as being animals, plants, protists, and simple organisms classed broadly as bacteria and viruses. Molecular studies (Williams et al. 2020; McGrath 2022) show that all living things evolved from a single last universal common ancestor (LUCA) perhaps 4000 Ma, and then split into two clades, Bacteria and Archaea. Eukaryota then branched from Archaea perhaps 2200 Ma, with some input from symbiotic bacteria to form fundamental cell organelles. The familiar plants, animals, and fungi are

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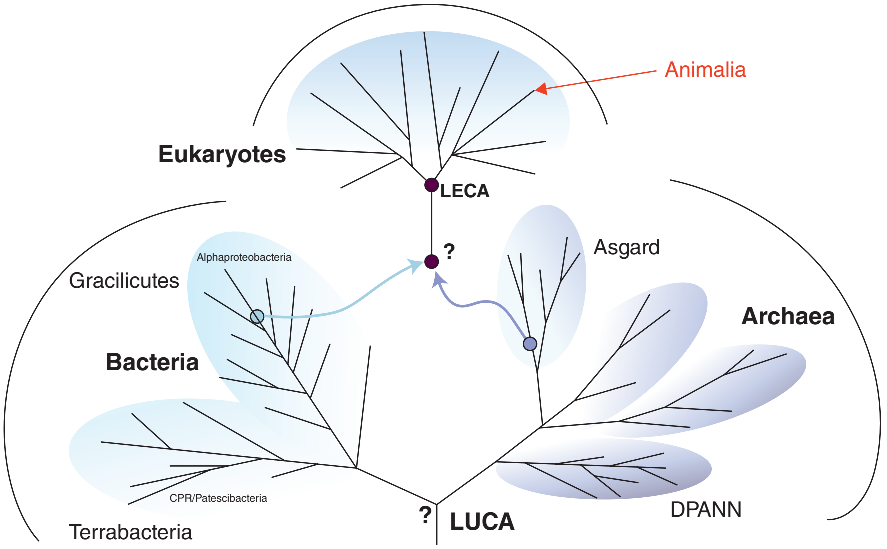


Figure 1.1 The Universal Tree of Life, showing the three main divisions: Eukarya, Archaea, and Eukaryotes. The last universal common ancestor (LUCA) and last eukaryote common ancestor (LECA) are marked. Animals are one of many twigs of the phylogenetic tree. *Source:* From McGrath (2022), reproduced according to CC BY-4.0 licence.

members of Eukaryota, all diagnosed by complex cells with a membrane-bound nucleus and mitochondria. Within Eukaryota are various protist groups, as well as plants, fungi, and animals, and of course, vertebrates are animals. Among animals, it has always been assumed that chordates are closely related to hemichordates (acorn worms and pterobranch worms) and echinoderms (starfish, sea lilies, and sea urchins), and this is now widely confirmed, based on morphological, developmental, and molecular evidence.

The purpose of this chapter is to explore the various lines of evidence that can be used to reconstruct the origin of the vertebrates: the study of modern animals that are vertebrate-like in some features, the study of molecular relationships, and fossils.

1.1 Sea Squirts and the Lancelet

There are two key groups of living non-vertebrate chordates: cephalochordates (amphioxus) and sea squirts. The amphioxus certainly looks superficially fish-like, but adult sea squirts look like rubbery little bottles, and so would hardly seem to be sensible candidates for close relatives of the vertebrates.

1.1.1 Cephalochordata: Amphioxus

The Cephalochordata (or Acraniata) have long been understood to be close relatives of the vertebrates. The clade includes 30–35 species that live generally in shallow seas of temperate to tropical latitudes, the most famous of which is the amphioxus or lancelet, *Branchiostoma*. The adult amphioxus is convincingly chordate-like, being a 50 mm-long paperknife-shaped animal that looks like a young lamprey or eel yet lacking a head (Bertrand and Escriva 2011; Holland 2015; Zhang et al. 2018; d'Aniello et al. 2023). Amphioxus swims freely by undulating its whole body from side to side, and it burrows in the sediment on the sea floor (Figure 1.2a, b).

Amphioxus feeds by filtering food particles out of the seawater. Water is pumped into the mouth and through the pharynx by cilia or the gill slits, and food particles are caught up in a bag of mucus produced by the endostyle, the feeding system seen also in tunicates and in the larvae of the lamprey. The mucus with its contained food particles is pulled into the gut for digestion, whereas the seawater passes through the gill slits into the atrium. Oxygen is also extracted, and the wastewater then exits through the **atriopore**.

The anatomy of amphioxus, with its pharynx, notochord, dorsal nerve cord, myotomes, and endostyle (Figure 1.2c) is typically chordate. Swimming and burrowing are done by means of lateral contractions of the myomeres acting against the stiff rodlike notochord.

1.1.2 Urochordata: Sea Squirts

A typical sea squirt, or tunicate, is *Ciona* (Figure 1.3a), which lives attached to rocks in seas around the world. It is a 100–150 mm-tall bag-shaped organism with a translucent outer skin (the tunic) and two openings, or siphons, at the top. *Ciona intestinalis* is one of about 3000 species of urochordates that mostly live in shallow seas, are generally attached to the seabed, and live either a solitary or colonial existence. But the adult sea squirts do not look much like vertebrates.

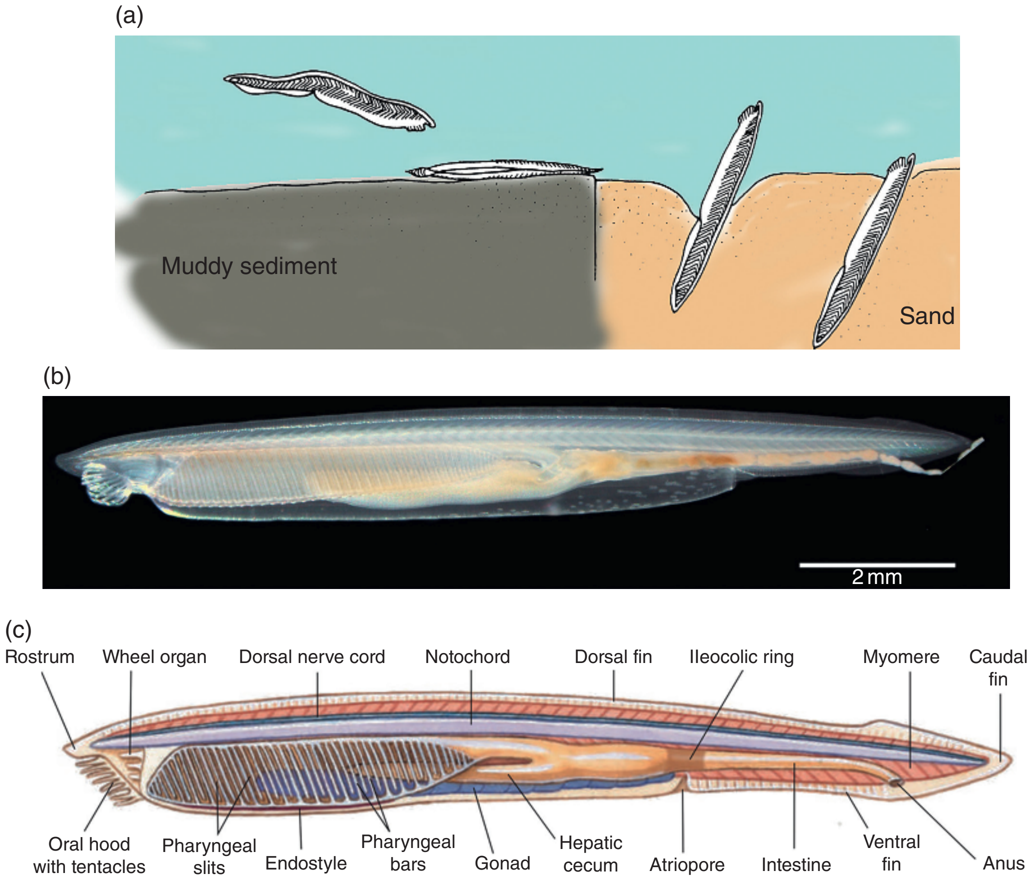


Figure 1.2 Amphioxus, a cephalochordate: (a) modes of life, including swimming and burrowing into sand for protection; (b) external appearance; (c) internal anatomy. *Source:* (a) Adapted from Pough et al. (2022) and other sources. (b) Campos-Dávila et al. 2019/Pensoft Publishers/CC BY 4.0. (c) Systematicist/Wikimedia/Licensed under CC BY 4.0.

The internal structure is fairly complex (Figure 1.3b). A large **pharynx** fills most of the internal space, and its walls are perforated by hundreds of gill slits, each of which bears a fringe of **cilia**, fine hair-like vibratile structures. Seawater is pumped through the inhalant siphon into the pharynx by beating movements of the cilia, and the water is then passed through a surrounding cavity, the **atrium**, and ejected through the exhalant siphon. The pharynx serves mainly to capture food particles from the stream of seawater that flows through it. The seawater is drawn into a filter bag of mucus, which is produced inside the pharynx by an organ called the **endostyle**. During feeding, the endostyle continuously secretes mucus into the **oesophagus**, together with the food particles that it has filtered from the seawater, and the food is passed to the stomach for digestion. Tunicates also have a heart that pumps the blood around the body; an intriguing aspect is that the heart stops beating every few minutes and the direction of blood flow reverses.

Why is *Ciona* identified as a chordate? The pharynx and other structures are in fact very like those of the cephalochordates and lamprey larvae, but further evidence is to be found in the **larval** stage, when the sea squirt is a tiny free-swimming tadpole-shaped animal with a head and a tail. The larval sea squirt (Figure 1.3c) has a notochord that runs along the tail, and this identifies it as a chordate. There are muscles on either side of the notochord that contract alternately, causing the tail to beat from side to side, and this drives the animal forward in the water. The larva has a dorsal nerve cord, running along the tail just above the notochord, and this expands at the front into a very simple brain that includes a light sensor (an 'eye') and a tilt detector.

The larva then settles on a suitable surface. It up-ends onto the tip of its 'snout' and attaches itself by means of adhesive suckers (Figure 1.2d). The notochord and tail portions wither away, and the pharynx and gut expand to fill up the body cavity. This extraordinary metamorphosis occurs rapidly to allow the adult to start feeding in its new way as soon as possible.

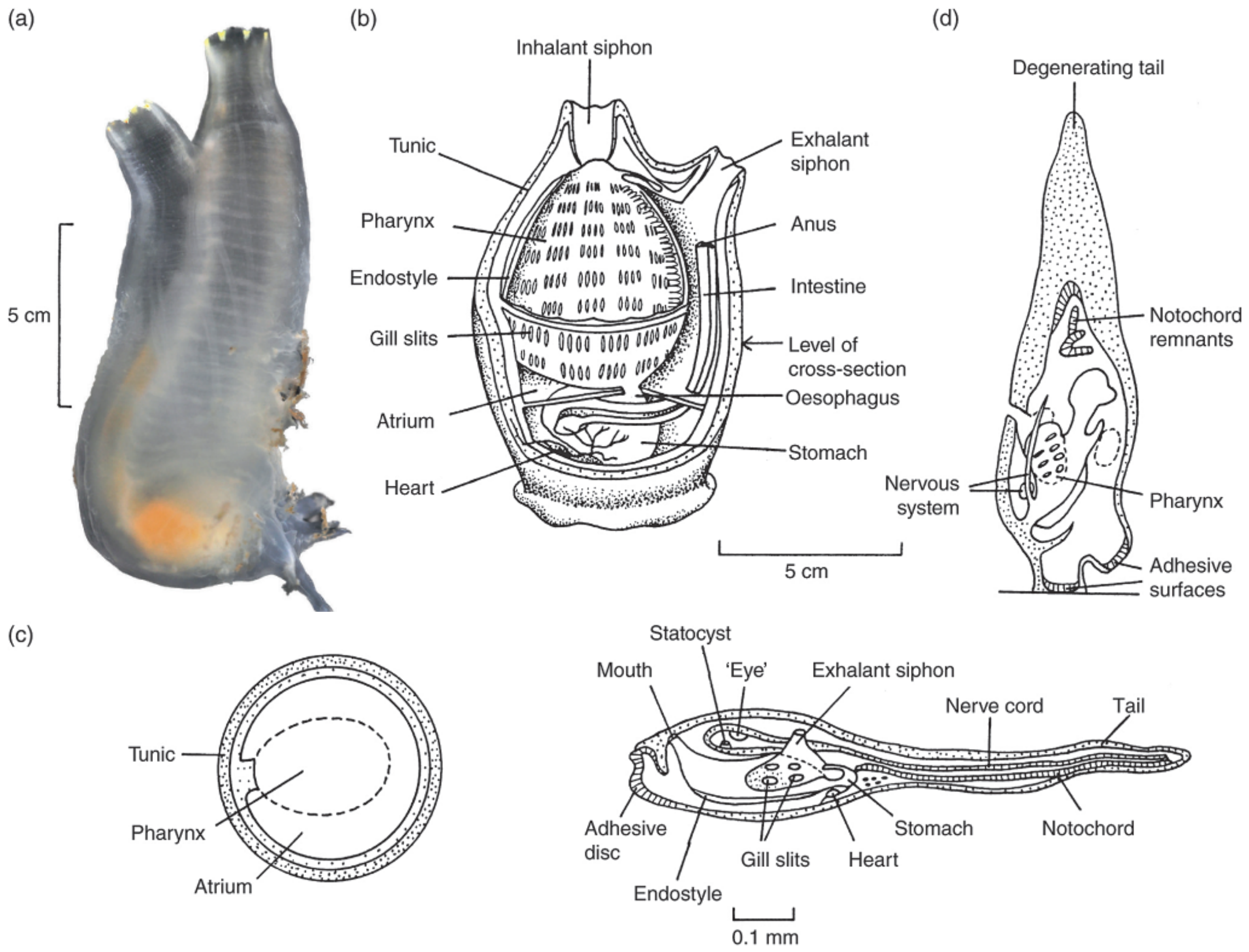


Figure 1.3 The sea squirts: (a) *Ciona*, external view; (b) internal anatomy and cross-section of an adult; (c) swimming larva; (d) metamorphosing form. *Source:* (a) E. A. Lazo-Wasem/Yale University/Wikipedia Commons/Public Domain. (b–d) Adapted from Jefferies (1986) and other sources.

1.2 Ambulacraria: Echinoderms and Hemichordates

Unexpected relatives of chordates are the Ambulacraria, a clade consisting of echinoderms and hemichordates. The living members of these groups do not look much like modern vertebrates, but there is considerable evidence for the relationship.

1.2.1 Echinodermata: Sea Urchins and Starfish

Echinoderms today include such familiar animals as starfish and sea urchins, as well as ophiuroids (brittle stars), crinoids ('sea lilies') and holothurians ('sea cucumbers'). There are some 7000 species of living echinoderms and 13,000 extinct species. Echinoderms all share four key features: (1) a calcite skeleton made from many ossicles, each composed of many aligned small crystals in a somewhat spongy arrangement called stereom; (2) a water vascular system that functions in locomotion, respiration, and feeding; (3) ossicles are linked by mutable collagen, ligaments that are normally rigid but can be loosened; and (4) pentaradial (fivefold) symmetry. Most of these special features of echinoderms do not show close similarities with either chordates or hemichordates, but the water vascular system may have evolved from simple tentacular systems, such as those of pterobranch hemichordates.

The first putative echinoderms include *Arkarua* from the Vendian of Australia, a disc-shaped organism with radial ridges and a five-pointed central depression, but it has no stereom or evidence of a water vascular system, and the identification is inconclusive. The first definitive echinoderms appeared in the Early Cambrian as part of the Cambrian Explosion, and these included some close relatives of living forms as well as other entirely extinct groups, some of them lacking pentaradial symmetry (Zamora et al. 2013; Nanglu et al. 2023a).

1.2.2 Hemichordata: Pterobranchs and Acorn Worms

The hemichordates (Kaul-Strehlow and Röttinger 2015; Tassia et al. 2016; Maletz 2019; Lowe 2021) include about 130 species belonging to two superficially very different kinds of marine animals. The first, the pterobranchs such as *Cephalodiscus* (Figure 1.4a, b), are small animals that live in loose colonies on the seabed in the southern hemisphere and in equatorial waters. *Cephalodiscus* has a plate-like head shield, a collar with five to nine pairs of feeding arms, and a sac-like trunk perforated by a pair of gill slits and containing the gut and gonads, and the body ends in a contractile stalk. Cilia on the arms produce a feeding current, and food particles are captured by mucus on the arms while water passes out of the pharynx through the gill slits. The animal lives in or around a group of horny tubes that the colony has constructed, and it attaches itself inside these tubes by means of a sucker on the end of the stalk.

The second hemichordate group, the acorn worms, or enteropneusts, such as *Saccoglossus*, are worm-like animals varying in length from 20 mm to 2.5 m. They live in burrows low on the shore in Europe and elsewhere. *Saccoglossus* (Figure 1.4c) has a long muscular proboscis that fits into a fleshy ring or collar behind. The mouth is placed beneath this collar, and seawater and sand are pumped through the gut and expelled through an anus at the posterior end of the body. The long body is pierced by small holes at the front end, homologous with the gill slits of *Cephalodiscus*, sea squirts, amphioxus, and vertebrates,

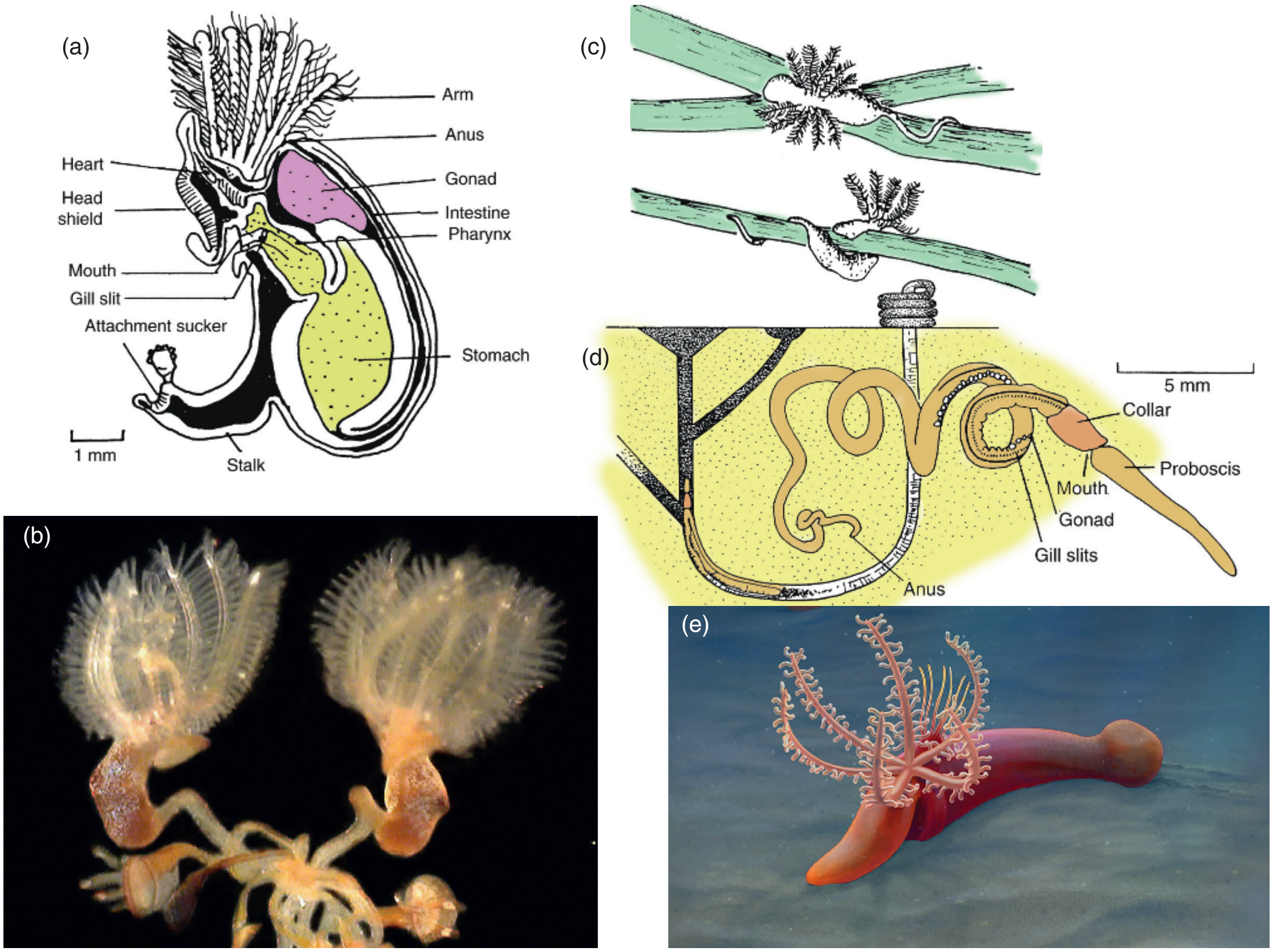


Figure 1.4 Typical hemichordates: (a–c) the pterobranch *Cephalodiscus*, internal anatomy, and mode of life; (d) the enteropneust *Saccoglossus*, mode of life, and external anatomy; (e) reconstruction of Cambrian hemichordate *Gyaltsenglossus*. *Source:* (a, c, d) Adapted from Jefferies (1986) and other sources. (b) Photo by Halanych Lab. (e) Emily S. Damstra/Wikimedia Commons/Public Domain.

based on morphology and expression of developmental genes (Cannon et al. 2013). Studies of development show that the postanal tail of *Saccoglossus* is not homologous with the tail of vertebrates (Stach and Kaul 2011).

The phylogeny of hemichordates is actively debated. However, morphological and molecular (Cannon et al. 2013; Lowe 2021) data concur that Hemichordata is a valid phylum. Hemichordates do not have a notochord at any stage, but they possess gill slits, as in chordates, and giant nerve cells in the nerve cord of the collar region that are probably equivalent to similar nerve cells in amphioxus and early vertebrates. Both pterobranchs and enteropneusts share morphological characters indicating monophyly of the Hemichordata, such as the stomochord (an anterior buccal tube on the dorsal part of the pharynx) and mesocoelomic ducts. Earlier molecular phylogenetic studies suggested that enteropneust worms were either monophyletic (based on 28S rDNA) or not (based on 18S rDNA), but micro-RNAs provide strong evidence for monophyly (Peterson et al. 2013; Peterson and Eernisse 2016).

With one exception, hemichordates have a limited fossil record because they generally lack hard parts. The exception refers to the extinct colonial graptolites, which are generally identified as a clade of hemichordates, and particularly allied with pterobranchs, based on similarities in the ultrastructure of their wall structures (Maletz 2019). For a long time, there were only rare fossils of the two extant hemichordate clades from the Carboniferous and Jurassic until reports (Caron et al. 2013; Maletz 2019; Nanglu et al. 2020) of Cambrian specimens from Chengjiang and the Burgess Shale. The worm-like *Spartobranchus* from the Burgess Shale shows a fibrous tube that might be a precursor of the pterobranch periderm, suggesting that pterobranchs arose from enteropneust-like ancestors. *Gyaltsenglossus*, also from the Burgess Shale (Figure 1.4e), is also elongate, 15–20 mm long, with six feeding arms, each bearing about 15 pairs of tentacles; it resolves the problem: it shows a combination of the elongate proboscis and elongate body of enteropneusts and the feeding arms of pterobranchs (Nanglu et al. 2020).

1.2.3 Ambulacrarian Origins

Echinoderms and hemichordates together form the clade Ambulacraria, and palaeontologists have sought evidence of ambulacrarian ancestors in the Precambrian and Cambrian. In particular, they have tried to understand whether any of the reported Vendian and Cambrian fossils identified as either Echinodermata or Hemichordata might in some way represent ancestors of both.

The best candidates are the Cambroernida, an assemblage of unusual, stalked and disc-like creatures. *Herpetogaster* from the Burgess Shale (Caron et al. 2010) looks like a pterobranch hemichordate with a segmented body, a stalk, and a pair of branching, feeding tentacles on its head. The eldoniids include about ten genera from the Cambrian and Ordovician of disc-shaped animals, measuring 30–50 mm across, and shaped like a jellyfish. Inside is a central cavity, perhaps the gut, and beneath are some branching feeding tentacles, as in hemichordates. A further example is *Rotadiscus* from the famous Early Cambrian Chengjiang Biota of China (see Box 1.1). These specimens (Li et al. 2023) show a double spiral structure, perhaps a chordate-like structure, as well as a horseshoe-shaped tentacle complex that resembles the tentacular systems of pterobranch hemichordates and echinoderms.

Based on study of *Rotadiscus* and other cambroernids, Li et al. (2023) reconstruct the ancestor as a bilaterally symmetrical chordate-like animal with a terminal anus and pharyngeal openings used for active suspension feeding, as in living cephalochordates, urochordates, and enteropneust hemichordates. This model differs from the traditional view of a sessile tentaculate ancestor.

It is still very uncertain whether there is such a clade as the Cambroernida, or whether the diverse species belong in different places in the evolutionary tree (Naglu et al. 2020). For example, the feeding tentacles have not been proven to be hemichordate-like and could resemble those of other filter feeders, such as brachiopods. The disc-shaped eldoniids have at times been interpreted as jellyfish or echinoderms.

1.3 Deuterostome Relationships

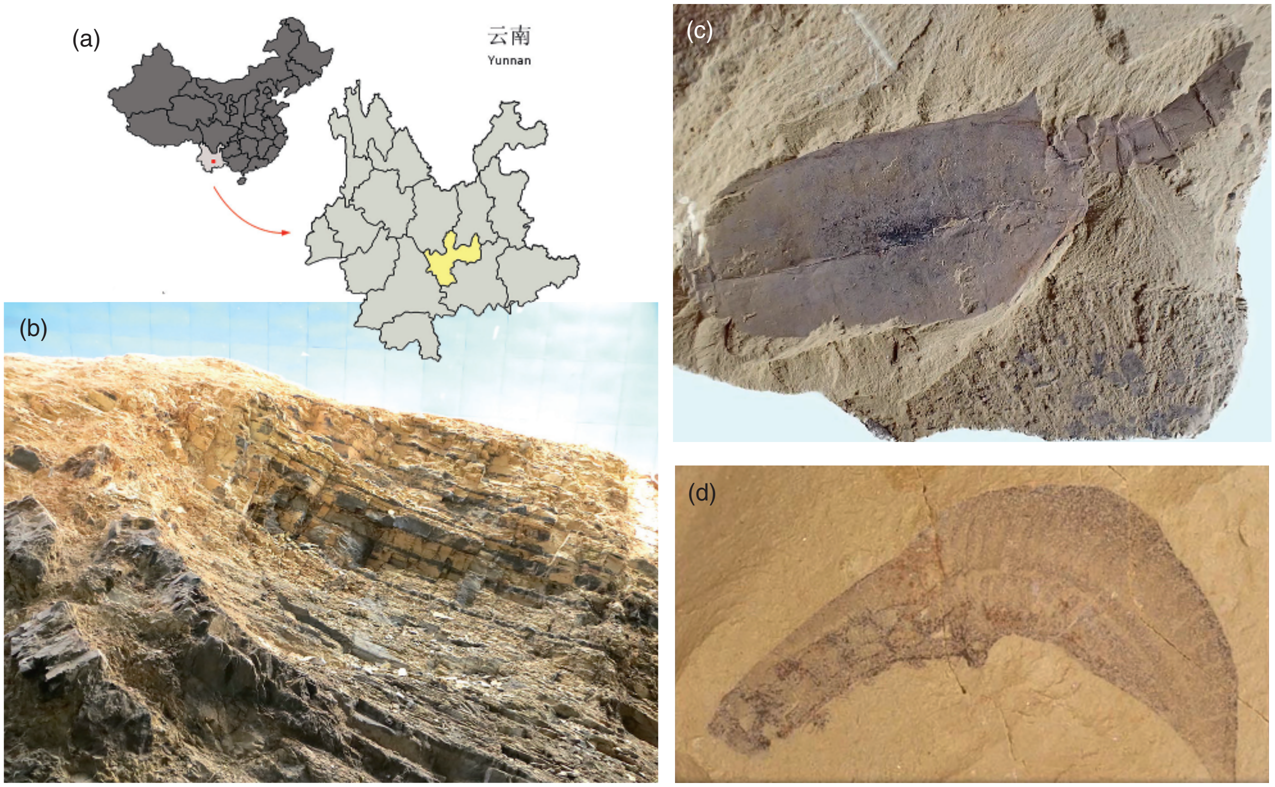
The relationships of chordates used to be rather problematic, but intensive studies of embryological and molecular data have provided a clearer picture in some ways, but not in others (see Section 1.3.2). The Phylum Chordata is part of a larger clade, the Deuterostomia, comprising chordates, hemichordates, and echinoderms. The ‘traditional’ view (Figure 1.5a) was to assume that hemichordates were the sister group to chordates because they both share ciliated gill slits and giant nerve cells, as well as other features that are not seen in echinoderms. Enteropneusts were sometimes said to be closer relatives of chordates since their gill slits are similar, they have a very short dorsal hollow nerve cord, and some other features of the gut not seen in pterobranchs. Most authors regarded amphioxus as the closest relative of the Vertebrata on the basis of 10–15 features that are not seen in tunicates.

Box 1.1 The Chengjiang Biota

The Chengjiang biota from Yunnan Province, south-west China, is exciting because it is one of the oldest sources of exceptionally preserved organisms, falling early in the great Cambrian radiation of animals in the sea (Shu et al. 2010; Zhao et al. 2013; Hou et al. 2017). The site was only discovered in 1984 but has revolutionized understanding and is celebrated as one of the great fossil localities of the world. The fossils come from different levels through several hundred metres of mainly fine-grained sediments, comprising the Maotianshan Shales (illustrations a, b). At first, the site was compared with the well-known Burgess Shale, a Middle Cambrian locality in Canada that has yielded numerous exceptionally preserved arthropods and the putative chordate *Pikaia*. Chengjiang, however, is at least 10 Myr older, dating from the Early Cambrian, some 518 Myr ago.

The Chengjiang biota is rich, having been collected now from over 30 localities that have produced tens of thousands of specimens. The fauna consists of more than 230 species, mainly of arthropods (trilobites and trilobite-like forms), sponges, brachiopods, worms, and other groups, including possible early deuterostomes, such as the vetulicolians and yunnanozoans, as well as the first fishes (Zhao et al. 2013). Some of the arthropods are like Burgess Shale animals, but others, such as the early deuterostomes (illustrations c, d), seem to be unique. Most of the animals lived on the bottom of the seabed, filtering organic matter from the sediment. There were a few floaters and swimmers, and some of the larger arthropods were clearly predators, feeding on the smaller bottom-dwellers.

The Chengjiang beds are grey mudstones that were deposited in a shallow deltaic environment, and the animals lived in oxygen- and nutrient-rich conditions where unstable salinity and high sedimentation rates were the main stressors (Saleh et al. 2022). As animals died, their carcasses were swept offshore by retreating storms and suffered little scavenging or microbial decay. The soft tissues may be preserved in exquisite detail, replaced by phosphate or by pyrite, and some as thin organic films. The grey sediment weathers on contact with the air to a light grey or yellow colour, and the fossils may also be grey, or sometimes reddish, and with internal anatomical details picked out in shades of grey, brown, and black.



Chengjiang and its fossils: (a) location in China and in Yunnan; (b) Maotianshan Shales in the on-site Chengjiang Museum; (c) the vetulicolian *Vetulicolia* (d) the yunnanozoan *Yunnanozoon*. *Source:* (a) Artwork by Croquant, Wikimedia. (b) Martin Smith/Wikimedia Commons/CC BY-SA 4.0. (c) Masahiro Miyasaka/Wikimedia Commons/CC BY-SA 4.0. (d) Zhu Maoyan/Wikimedia Commons/Public Domain.

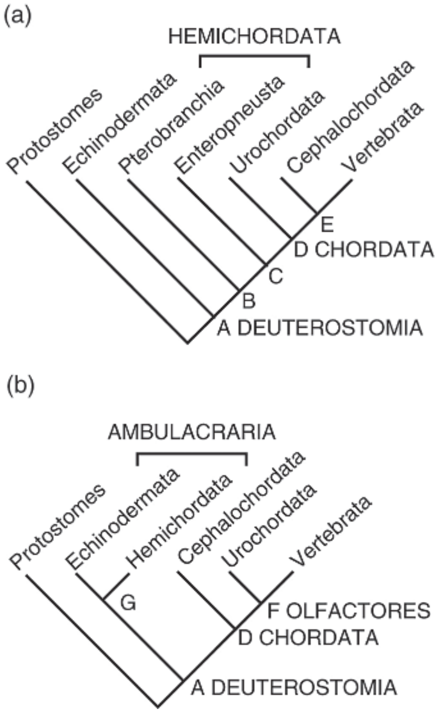


Figure 1.5 Cladograms showing the relationships of the main deuterostome groups: (a) the 'traditional' model, and (b) the molecular model. *Source:* Based on various sources.

The current view (Figure 1.5b) is very different but is supported by extensive morphological and molecular data (Swalla and Smith 2008; Edgecombe et al. 2011; Cannon et al. 2013; Dunn et al. 2014; Telford et al. 2015; Peterson and Eernisse 2016; Li et al. 2019; Nanglu et al. 2023a). Hemichordates are paired with echinoderms as the clade Ambulacraria, and within the clade Chordata cephalochordates are the earliest diverging clade, and Urochordata the nearest sister group of Vertebrata, as clade Olfactores because of shared characters in the olfactory region.

The clade Deuterostomia is part of a clade of all the bilaterally symmetrical animals, the Bilateria, and these in turn fall within Metazoa, the animals. But what exactly diagnoses the Deuterostomia and how can some of our closest relatives be sea urchins, starfish, and wormlike animals? The answers come from **embryology**, which is the study of the early phases of development from fertilization of the egg to the point of birth, and from molecular phylogenetic analysis.

1.3.1 Embryology and the Position of the Anus

In early development each animal starts as a single cell. Soon this cell begins to divide, first into 2 cells, then 4, then 8, 16, and so on (Figure 1.6a). Eventually, a hollow ball of cells is produced, called the **blastula** stage. A pocket of cells then moves inwards, forming the precursor of the gut and other internal structures. The opening of this deep pocket is called the **blastopore**. You can imagine pushing in the walls of a hollow rubber squash ball with your thumb to produce a model of this embryonic pattern, known as the **gastrula stage**.

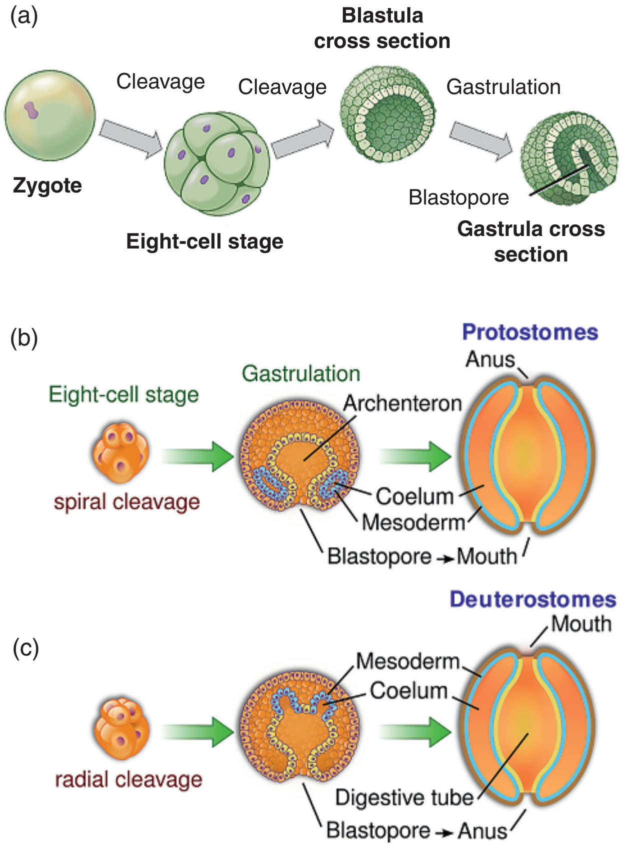


Figure 1.6 Embryonic development: (a) sequence of cell division in a typical animal, from the single-cell stage, through the blastula stage, to the gastrula stage. (b) Fate of the blastophore in protostomes, and (c) in deuterostomes. *Source:* (a) CNX Openstax/Wikimedia/Licensed under CC BY 4.0. (b, c) YassienMrabet/Wikimedia/Licensed under CC BY 4.0.

Embryologists noticed some time ago that animals fall into two large groups depending on the relative orientation of the mouth and anus. The classic story is that in most invertebrates (the **protostomes**), the blastopore becomes the mouth (Figure 1.6b), whereas in others (the **deuterostomes**), including the chordates, this opening becomes the anus (Figure 1.6c), and the mouth is a secondary perforation. Such a dramatic turnaround, a switch from mouth to anus, seems incredible. Note, however, that many protostomes show deuterostomy, and this condition may be shared by all Bilateria (Peterson and Eernisse 2016). This peculiarity of embryological development was noted over a century ago, and the group Deuterostomia was named in

1908; but does it stand up to the scrutiny of modern molecular phylogenetics?

1.3.2 Animal Phylogenomics

It might seem obvious what is an animal, and what is not. All creatures from sponges and corals to crabs, clams, and birds, are animals, members of the clade Metazoa; animals are diagnosed by a combination of feeding, being motile, lacking rigid cell walls, and passing through the blastula embryonic stage. However, these characteristics are not all exclusive. First, metazoans are distinguished from most plants and algae by being **heterotrophs**, meaning they feed on other organisms to acquire carbon, which is digested in an internal chamber (gut), whereas plants and algae are able to fix carbon from the atmosphere or water. However, fungi and many bacteria are also heterotrophs. Secondly, metazoans are **motile**, meaning they use energy to move spontaneously and actively, at least at some stage in their lives (larval stages in 'fixed' forms such as sponges and corals can swim), although some bacteria and protists are also motile, moving by means of a flagellum. Thirdly, animals lack the rigid cell walls seen in plants, fungi, and algae, and fourthly, most pass through the blastula embryonic stage (see Section 1.3.1).

Metazoa, Bilateria, and Protostomia are **monophyletic** groups or **clades**. A clade is a group that has a single common ancestor, and that includes all the descendants of that ancestor (see Section 2.5.1). Before the advent of molecular phylogenetics (see Section 2.5.2), and even after, there has been active debate about the relationships of the various animal clades. It is usually easy to determine the membership of these major clades, the phyla (see Box 2.4) – such as arthropods, molluscs, or sponges – but determining how the phyla relate to each other within Metazoa has been difficult. However, by 2010, a consensus about the major outlines of animal relationships had been reached (Figure 1.7).

The fundamental division of Metazoa distinguishes six early branching clades (including sponges and corals) from the Bilateria, supported by both morphological and molecular evidence (Edgecombe et al. 2011; Dunn et al. 2014; Telford et al. 2015; Peterson and Eernisse 2016; Marletaz et al. 2019; Laumer et al. 2019). The Bilateria had bilateral symmetry from the start, and most are **triploblastic**, meaning they have three fundamental body wall tissues that arise from the ectoderm, mesoderm, and endoderm in the embryo. Non-bilaterian metazoans may be diploblastic, lacking the mesoderm, or monoblastic like sponges and placozoans. Within Bilateria, most animals are Nephrozoa, taxa that are characterized by the possession of an excretory system. Finally, Nephrozoa is divided into two major clades, Protostomia and Deuterostomia, long recognized on embryological grounds. Protostomes include the Ecdysozoa (animals that moult, such as nematodes, arthropods, priapulids, and some minor groups) and Spiralia (animals with spiral development, such as bryozoans, annelids, molluscs, brachiopods, rotifers, and other phyla). Most spiralians belong to the clade Lophotrochozoa.

The monophyly of Deuterostomia is confirmed by morphology and development, but results from molecular data are debated. All deuterostomes share the posterior blastopore that generally becomes the anus, as well as gill slits (present only in precursors of the echinoderms). Many molecular phylogenetic analyses support monophyly (e.g. Swalla and Smith 2008; Edgecombe et al. 2011; Röttinger and Lowe 2012; Cannon et al. 2013; Peterson and Eernisse 2016), but more recent work has raised a debate, and this has a lot to do with whether Xenacoelomorpha are included within Deuterostomia or not; this obscure clade includes the Xenoturbellida and Acoelomorpha, simple worms with no through gut and a simple nervous system as deuterostomes. If they are deuterostomes, then Kapli et al. (2021) have shown that molecular data provide only quite weak support for the monophyly of Deuterostomia, which means that the evolutionary branch at the base is very short, or that the ancestral bilaterian was deuterostome-like, making Deuterostomia, as we currently define it, paraphyletic. On the other hand, Juravel et al. (2023) show that Deuterostomia is reasonably well supported by certain molecular data, especially if Xenacoelomorpha is not included, but is placed as sister to the other bilaterians.

1.4 Chordate Origins

Among morphological characters, the chordates all share several unique features, such as a notochord, a dorsal hollow nerve cord with a shared developmental pattern, an endostyle organ (equivalent to the thyroid gland of vertebrates), and a tail used for swimming. It is generally accepted that only chordates have true tails. A tail technically may be defined as a distinct region extending behind the visceral cavity and specifically located entirely behind the anus; hence the term 'postanal tail'. Non-chordates, such as insects, worms, molluscs, jellyfish, and sea urchins, do not have tails. What of the fossil evidence?

There are many putative early fossil chordates, and their numbers have grown hugely since 1995, with the announcement of remarkable new finds from the Chengjiang biota of China, an Early Cambrian deposit. These new specimens, combined with studies of modern forms, give clues about the early evolution of chordates and vertebrates, but there are many debates (Peterson and Eernisse 2016; Li et al. 2023; Nanglu et al. 2023a, b). In interpreting the fossils, it's important to keep in mind the key characteristics of the earliest vertebrates.

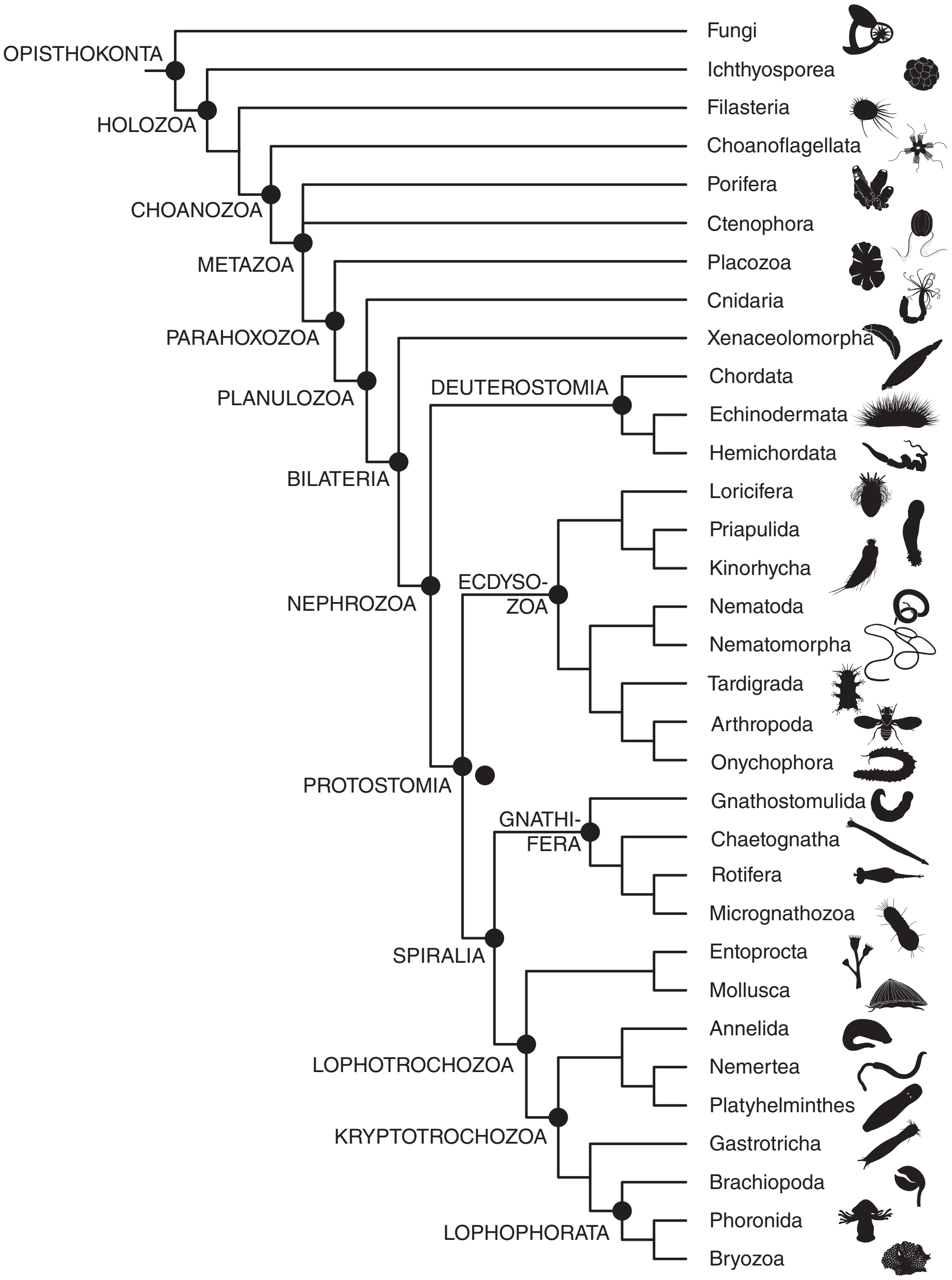


Figure 1.7 Relationships of the major phyla of animals plus their unicellular and multicellular relatives based on accumulated knowledge from anatomy and embryology, combined with current phylogenomic work. *Source:* Courtesy of Phil Donoghue, drawing on Marletaz et al. (2019) with silhouettes from Phylopic, by Thibaut Brunet, Guillaume Dera, Ludwik Gasiorowski, Steve Haddock, Scott Hartman, Michael Keesey, Riccardo Percudani, Thea Rogers, Noah Schlottman, and Yan Wong.

1.4.1 Diverse Early Chordates

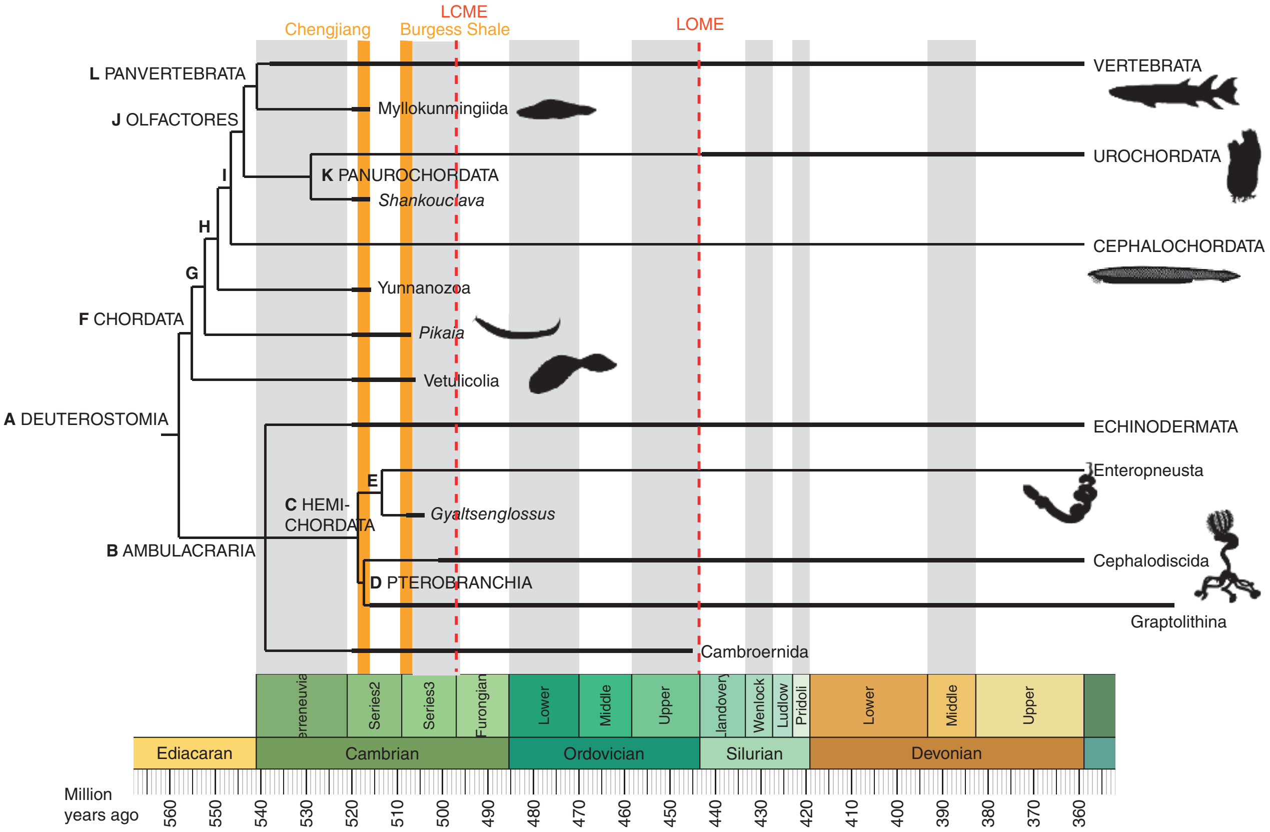
There are five main categories of early chordate fossils: possible urochordates, possible cephalochordates, vetulicolians, *Pikaia*, and yunnanozoans (Nanglu et al. 2023a), and these can be placed in the phylogenetic tree according to their anatomical features (see Box 1.2). At one time, conodonts, represented in the fossil record generally only by their tooth elements, were treated as dubious chordates. Conodonts are now placed firmly within the Vertebrata, as jawless fishes, as are some of the early chordate taxa from Chengjiang, such as *Haikouichthys* (see Chapter 3).

The fossil record of cephalochordates is poor. The Chengjiang biota includes a superficially amphioxus-like

Box 1.2 Deuterostome Phylogeny

In attempting to understand the relationships among the various Cambrian deuterostome fossils, the phylogenetic tree of living forms, based on molecular and morphological data (Swalla and Smith 2008; Edgecombe et al. 2011; Cannon et al. 2013; Dunn et al. 2014; Telford et al. 2015; Peterson and Eernisse 2016; Li et al. 2019; Nanglu et al. 2023a) is the basis (Figure 1.1). Among living forms, hemichordates are paired with echinoderms as the clade Ambulacraria, and within the clade Chordata cephalochordates are the earliest diverging clade, and Urochordata the nearest sister group of Vertebrata, as clade Olfactores because of shared characters in the olfactory region.

As described in the text, tens of fossil species have been assigned to a variety of positions either at the base of Deuterostomia, Ambulacraria, or Chordata, or as members of the clades Hemichordata, Cephalochordata, or Urochordata. Nearly all such fossils have been allocated multiple different locations in the phylogeny according to different authors, and we show the current consensus view here (Tian et al. 2022; Li et al. 2023; Nanglu et al. 2023a, b). There is still a great deal of uncertainty about the nature and phylogenetic position of the Cambroernida, and the sequencing and positions of Vetulicolia, *Pikaia*, and Yunnanozoa, with new studies of key characters potentially moving *Yunnanozoon* up towards Vertebrata. See Box 3.1 for phylogeny of Vertebrata.



Cladogram showing the relationships between the living deuterostome clades and the key fossil taxa. Tree shape and apomorphies are from Swalla and Smith (2008), Cannon et al. (2013), Tian et al. (2022), and Nanglu et al. (2023a). Synapomorphies: **A DEUTEROSTOMIA**, blastopore becomes anus during development, pharyngeal gill slits, gill openings; bipartite mesocoel, mesocoelomic ducts; **B AMBULACRARIA**, trimeric arrangement of the adult coelom, axial complex with hydropore, dipleureula larva with neotroch; **C HEMICHORDATA**, stomochord, proboscis, heart-kidney complex; **D PTEROBRANCHIA**, cephalic shield; **E,** nuchal skeleton, pre-oral ciliated organ; **F CHORDATA**, notochord present and not attached to gut, dorsal hollow nerve cord with neural-plate stage in development, endostyle organ; **G**, tentacles round mouth; **H**, pharyngeal gill slits, branchial arches consist of cellular cartilage; extracellular matrix of cartilage dominated by microfibrils, possible brain, possible eyes; **I**, post-anal tail; **J OLFACTORES**, specialized olfactory areas in buccal cavity, hind-tail tripartite, dorsal longitudinal canal connected with notochord; **K PANUROCHORDATA**, tunic, pharyngeal basket, oral siphon; **L PANVERTEBRATA**, calcium phosphate skeleton; neural crest; head, cranial skeleton, brain, and cranial nerves. Abbreviations: Lud, Ludlow; Pr, Pridoli; Wen, Wenlock. Vertical dashed red lines correspond to the Late Cambrian mass extinction **(LCME)** and the Late Ordovician Mass Extinction (**LOME**). *Source:* Silhouettes from Phylopic, digitized by Guillaume Dera, Scott Hartman, kaz079, T. Michael Keesey, and Yan Wong.

cephalochordate, *Cathaymyrus*, as well as the yunnanozoans, which were once identified as cephalochordates, but are now included among Chordata (see below). In the absence of hard tissues such as bone, these nonvertebrate chordates are not often preserved.

Tunicates (urochordates) have a patchy fossil record. Isolated impressions of sac-like bodies, and **trace fossils**, markings made in or on the sediment by the activities of animals, have been ascribed to tunicates. The best fossils are small sac-like specimens from Chengjiang, *Shankouclava*, which show a large perforated branchial basket, branchial slits, and an elongate endostyle (Chen et al. 2003). There is also a possible degenerating tail, suggesting this might be a larva that had just settled. A second early urochordate, from the Middle Cambrian of Utah, USA (Nanglu et al. 2023b), has a 32 mm tall and 19 mm wide barrel-shaped body with two long siphons and prominent longitudinal muscles (Figure 1.8a, b). This is like the adult stage of modern tunicates such as *Ciona* (Figure 1.3a), suggesting that the earliest tunicates had a two-phase life cycle consisting of a swimming larva and attached, sessile adult.

1.4.2 Vetulicolia

The Vetulicolia are an unusual group, based on about 20 species of *Vetulicola*, *Xidazoon*, *Didazoon* and others from the Chengjiang Formation, as well as *Banffia*, named in 1911 from the Burgess Shale in Canada, and only later associated with the Chinese fossils, and materials from the Cambrian site, Sirius Passet, in Greenland, and from the United States (Figure 1.8c, d). These animals look like sausage balloons, knotted in the middle: the body is in two parts, with bulbous sections in front of, and behind, a flexible connection. There is a large mouth with a strengthened rim and preserved internal structures include the

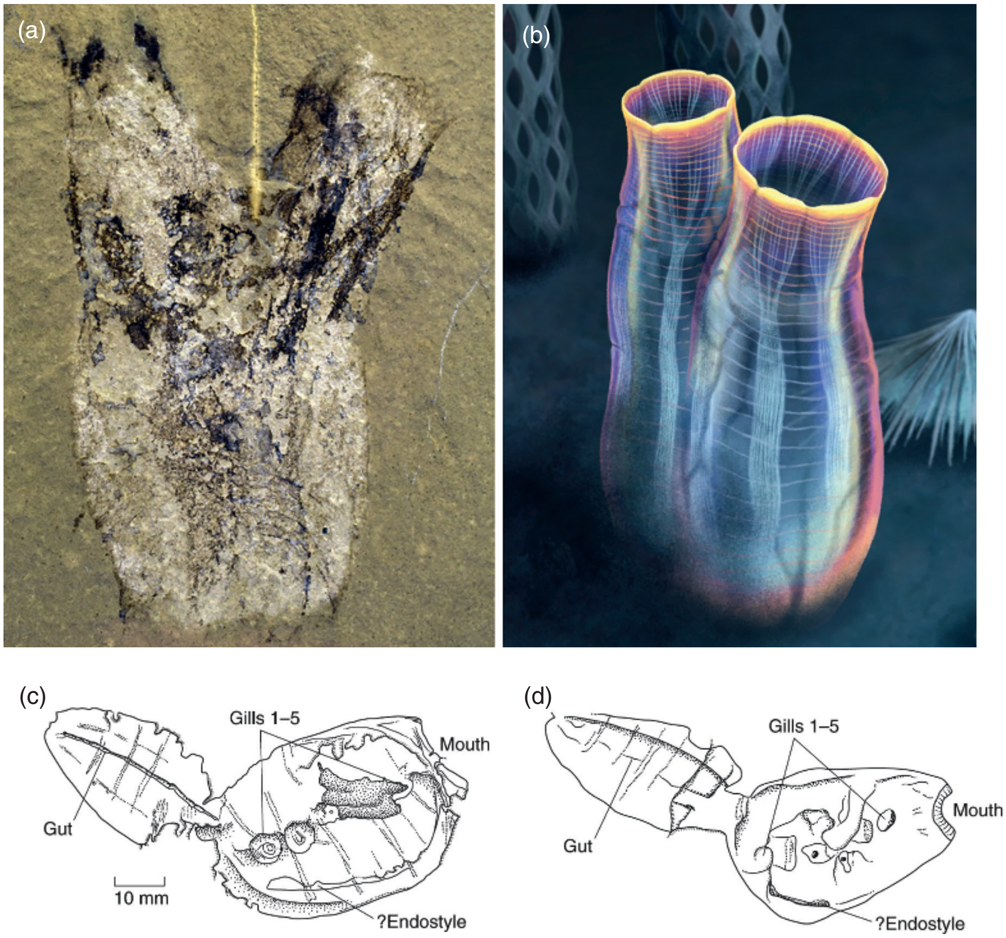


Figure 1.8 Early deuterostomes: (a, b) the Cambrian sea squirt *Megasiphon*, fossil and life reconstruction; (c, d) the vetulicolians *Didazoon* (c) and *Xidazoon* (d), showing how the body is divided into two sections that are joined by a flexible connection. *Source:* (a, b) Photograph by James C. Weaver; artwork by Franz Anthony; courtesy of Javier Ortega-Hernández, Harvard University, Cambridge, MA. Reproduced with permission. (c, d) D. Shu, Northwest University, Xi'an, China. Reproduced with permission.

guts. Both parts of the body appear to be crossed by transverse bands, and the tail is distinctly segmented.

On the mouth-bearing segment, presumably the front part of the body, are five circular structures in a row that have been interpreted as pharyngeal gill slits, capable of opening and shutting. This would have enabled the vetulicolian to swim around with its mouth open and gill slits shut, then open the gill slits and shut the mouth to expel the water, while extracting oxygen and food particles (Ou et al. 2012).

Vetulicolians have been identified as arthropods, deuterostomes, or chordates, but most researchers now agree on one of the last two. Evidence that vetulicolians are deuterostomes includes the gill slits and a possible endostyle, although the latter identification has been questioned. In vetulicolians, the intestine appears to terminate at the end of the body, so they may lack a postanal tail, a key chordate character. The nature of the gill slits and the presence or absence of a pharynx are debated. Some researchers have explicitly compared them to sea squirts and explained the supposed absence of the notochord by the suggestion that, as in sea squirts, the larval forms had a notochord, but it was lost in the sessile, bag-like adults. However, if they truly have a notochord, they are chordates.

1.4.3 *Pikaia*

The final early chordate to consider is *Pikaia* from the Burgess Shale in Canada, named in 1911 as an annelid, but subsequently widely regarded as an early chordate or even an early vertebrate (Figure 1.9). In a redescription of 114 specimens, Conway Morris and Caron (2012) highlight its chordate characteristics: a laterally compressed, hydrodynamic body with about 100 myomeres, a thin dorsal fin, a small bilobed head with tentacles but no eyes, possible pharyngeal pores, a pharyngeal cavity, an almost terminal mouth, a probable terminal anus (and hence no postanal tail), a dorsal nerve cord, a possible notochord, and a blood vascular system (Figure 1.9b). As with the yunnanozoans, however, determining the phylogenetic position of *Pikaia* is problematic.

Older than *Pikaia* is *Cathaymyrus*, a 22 mm-long, eel-shaped animal from the Chengjiang Biota (Shu et al. 1996). The head is missing, but there are structures interpreted as pharyngeal gill slits, a possible notochord, and myotomes. Both *Cathaymyrus* and *Pikaia* were identified as possible fossil cephalochordates, but they are more generally classed as basal chordates, close to the yunnanozoans (Conway Morris and Caron 2012; Nanglu et al. 2023a). *Pikaia*, like several other fossil chordates and vertebrates from Chengjiang and the Burgess Shale probably lived in large shoals as active, swimming plankton feeders (Figure 1.9c).

1.4.4 Yunnanozoa

The yunnanozoans, also from Chengjiang, such as *Yunnanozoon* and *Haikouella* (Figure 1.10a) are 25–40 mm long, and preserved as flattened bluish-grey to black films on the rock (Box 1.1d). They look like convincing examples of early chordates, perhaps even close to vertebrates, with their fishlike form, dorsal fin, postanal tail, and postulated notochord, gill slits, and head structures, but their phylogenetic position has been debated. One team identified these animals first as possible cephalochordates, and then upwards as vertebrates (Chen 2008), whereas the other team preferred to regard the yunnanozoans first as hemichordates, and then downwards as early deuterostomes allied to the vetulicolians (Shu et al. 2010). The problems revolve around different interpretations of coloured blobs, lines, and squiggles in the fossils. There are plenty of fossils – literally thousands – but anatomical interpretation is critical, and the way in which researchers labelled particular enigmatic structures in the fossils could bias the interpretation (Donoghue and Purnell 2009).

Representing the conservative viewpoint, Cong et al. (2015) recommended caution in identifying features such as a notochord, gill slits, segmented muscles, a large brain, or lateral eyes. For example, they note that the supposed notochord might be a dorsal nerve cord, the gill slits more like those of vetulicolians than vertebrates, and the muscle blocks more like segmentation of the body than vertebrate-like myomeres. They also note that the evidence identified by others as indicating a brain and paired sensory organs such as eyes is dubious, so removing them from close relationship to vertebrates. Nanglu et al. (2023a) also ranked yunnanozoans as basal Chordata, in an unresolved phylogenetic position beside vetulicolians.

On the other hand, new evidence identifies yunnanozoans unequivocally as vertebrates. Tian et al. (2022) reported that the seven branchial arches (Figure 1.10b) are composed of cellular cartilage within an extracellular matrix of microfibrils, which is a vertebrate-specific combination of tissue types. The researchers used X-ray microtomography, scanning and transmission electron microscopy, and energy dispersive spectrometry element mapping to study these fine details (the gills are spaced 1–2 mm apart) in large numbers of specimens. The new images confirm that the branchial arches are bamboo-like bars that stand nearly vertically with horizontal bars at both ends and slender gill filaments stretch out on either side of the vertical bars (Figure 1.10b). Each branchial bar consists of about 25 disc-like structures stacked one on top of the other, each of which is subdivided into several cellular chambers. These match precisely the size, shape, and arrangement of

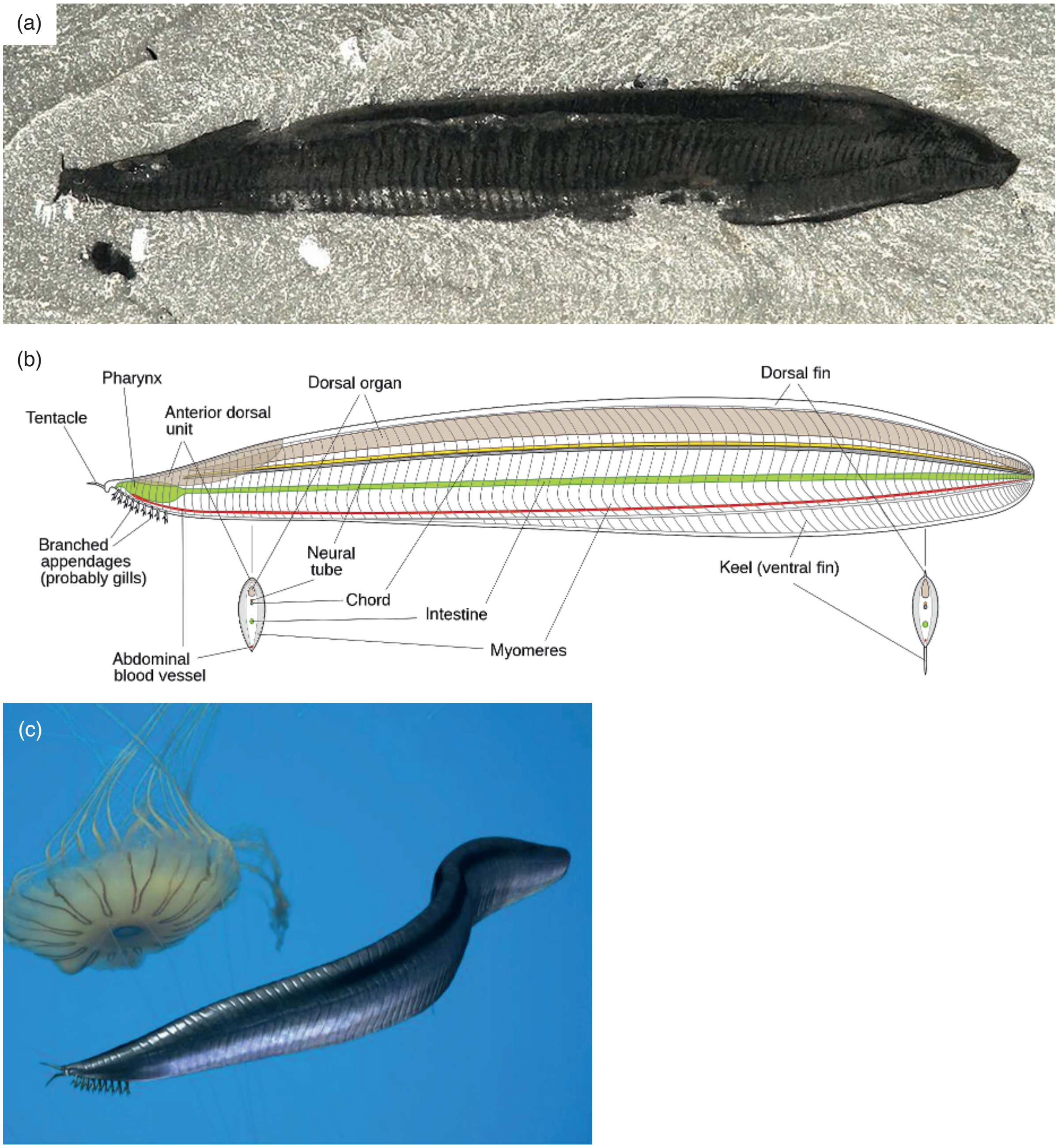


Figure 1.9 The early chordate *Pikaia* from the Burgess Shale: (a) Specimen in the U.S. National Museum; (b) anatomical interpretation; (c) life reconstruction. *Source:* (a) J.-B. Caron, Smithsonian Institution. Reproduced with permission. (b) Image redrafted from Conway Morris and Caron (2012) by Alexander Shatulin, Wikimedia. (c) Nobu Tamura/Wikimedia Commons/CC BY-SA 4.0.

chondrocytes, cartilage-forming cells, in the branchial arches of the embryos of modern vertebrates.

The yunnanozoan branchial arches also contain microfibrils just like those seen in the extracellular matrix of cartilage in lampreys and hagfish, as well as in cephalochordates. The new work confirms that the pharynx and gill slits of yunnanozoans (Figure 1.10c) functioned in active respiration and feeding, as in early vertebrates, suggesting that these really are close to vertebrates in their anatomy (Figure 1.10d).

This example shows how closer study, sometimes using new analytical tools and procedures, can reveal crucial information about anatomy of ancient chordate fossils. In this case, the new work on yunnanozoans has revealed new features of their pharynx and has had the effect of shifting them up the phylogenetic tree towards Vertebrata. Does new

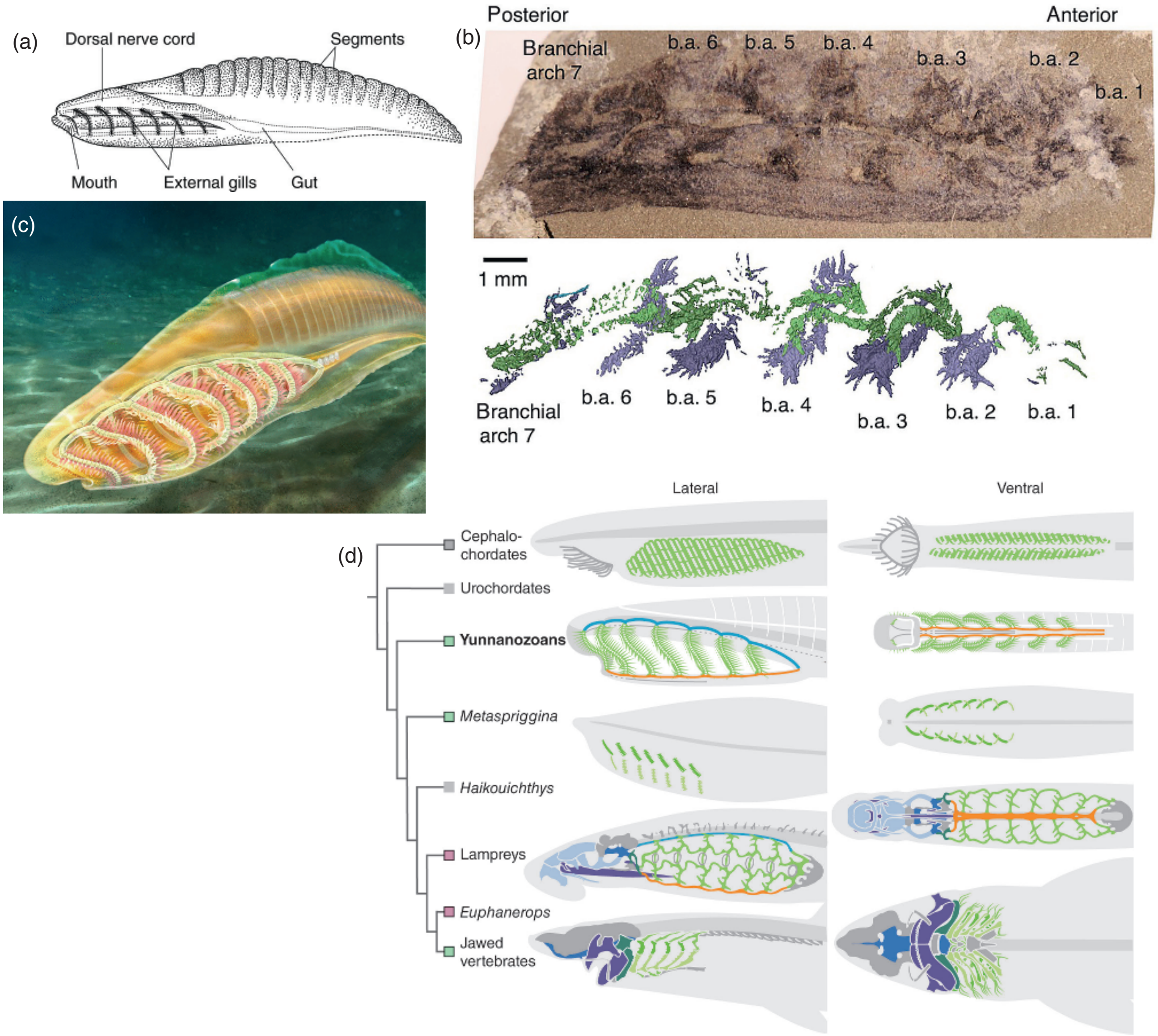


Figure 1.10 Anatomy of the yunnanozoans: (a) overview of main organ systems; (b) pharyngeal anatomy in specimen (above) and CT scan image (below), showing a lateral view of the seven branchial arches from the right-hand side, with left-hand arches beneath; (c) life reconstruction with cutaway showing branchial arch system; (d) phylogenetic tree comparing the pharyngeal skeleton of modern and fossil chordates. *Source:* (a) Degan Shu, Northwest University, Xi'an, China. (b, d) Qingyi Tian and Baoyu Jiang, Nanjing University, China. (c) Dinghua Yang and Fangchen Zhao, Nanjing Institute of Geology and Palaeontology of the Chinese Academy of Sciences, China. All reproduced with permission.

information like this always move fossils up the tree, or could it move them down? Studies in fossil decay (see Box 1.3) suggest that upward phylogenetic movement might be the norm.

1.5 Vertebrate Origins: Worm or Bag?

Are we any closer to identifying the original vertebrate – worm or bag? The fossils could indicate a 'bag-like' ancestor based on early urochordates like *Megasiphon* and some cambroernids, with the suggestion that their larvae were more fish-like. On the other hand, little critters like *Pikaia* and the yunnanozoans are perfect worm-like vertebrate precursors. The evidence of phylogeny (see Box 1.2) could go either way: our closest sister group, the Urochordata, are biphasic and bag-like as adults, and worm-like as larvae. Next closest living relatives, the Cephalochordata, are worm-like as adults, so was that their ancestral state or not?

The switch between larva and adult has been a popular idea, dating back 100 years, and it is worth exploring

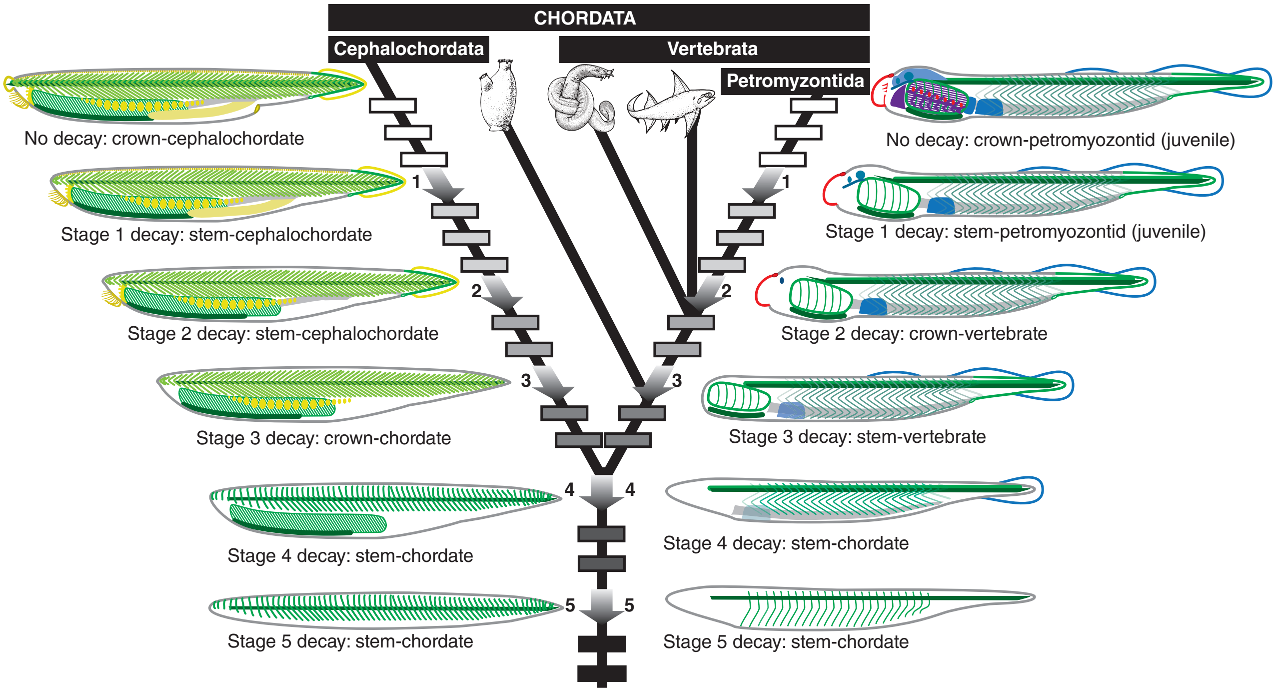
Box 1.3 Rotting Bias

When an organism dies its carcass decays, and information is lost. Until recently, such loss of information was assumed to be random, but taphonomic experiments on modern amphioxus and lampreys (Sansom et al. 2010) show that the first tissues to rot away take with them key diagnostic characters. In fact, through the process of decay over a few weeks, tissues are lost in such a way that the specimens become more and more generalized in appearance.

The rather smelly experiments on lamprey and amphioxus juveniles were run for up to 200 days, with dead specimens decaying in normal seawater and at reasonable temperatures. Tissues began to be lost quickly. In the case of amphioxus, the eye spot was lost after 11 days, the atriopore after 15, the anterior bulb after 21, and the midgut caecum and storage organ after 28. Most resilient to decay were the myomeres and the notochord, and before those the endostyle, pharyngeal arches, and gonads. Sansom et al. (2010) noted that these last tissues are the ones that are commonly seen in exceptionally preserved early chordate and deuterostome fossils from the Chengjiang and Burgess Shale biotas.

The initial suite of characters that disappeared in the decaying amphioxus specimens were diagnostic of Cephalochordata, whereas the myomeres and notochord are the most general chordate characters. Decay processes seem to favour the preservation of general characters, and phylogenetic analysis of chordate fossils will position the fossils in a lower position in the tree than is correct. Perhaps the fossil record of non-vertebrate chordates is affected by a systematic bias of stem-ward slippage down the cladogram, and many Cambrian chordate fossils may be placed too deep in the phylogeny. These experiments partly explain why palaeontologists have had such a hard time in finding the diagnostic characters that would help them to identify the true phylogenetic positions of vetulicolians, *Pikaia*, yunnanozoans, and early vertebrates such as *Haikouichthys* (see Chapter 3).

Parry et al. (2018) accepted that different tissues decay at different rates, but they noted that the processes are complex. In the decay experiments, for example, the notochord survives for a long time whereas nervous systems disappear early, and yet in fossils nervous systems and blood vessel systems are sometimes preserved and notochords have been hard to identify in many Cambrian organisms.



Morphological decay stages of amphioxus (left) and larval lamprey (right) and the phylogenetic position of each stage if interpreted as a fossil. Rectangles on branches of the phylogeny are morphological characters, their shade indicating the order of loss (white, early; dark, late). As each organism decays, its phylogenetic position moves down the tree; this is evidence of taphonomic bias in the identification of fossil chordates. Characters are colour-coded according to the hierarchical level for which they are informative (green, chordate; yellow, cephalochordate; blue, vertebrate; purple, cyclostome and vertebrate; red, petromyzontid). *Source:* Adapted from Sansom et al. (2010).

this model first before we look at evidence from modern evo-devo.

1.5.1 Tadpole Origins

The classic theory for the origin of vertebrates was that we arose ultimately from the sea squirt tadpole. In the 1920s, the distinguished zoologist Walter Garstang noted the similarities between the larval sea squirt (see Figure 1.3c), adult amphioxus (see Figure 1.2b, c), and vertebrates. The sea squirt tail seemed to him to be a transient appendage that evolved as an outgrowth from the body to ensure wide dispersal of the larvae before they settled. Garstang (1928) proposed that the evolutionary link between the sea squirts and all higher chordates is through a process termed **paedomorphosis**, the full development of the gonads and reproductive abilities in an essentially juvenile body. According to his view, an ancient sea squirt larva failed to metamorphose and became adult (i.e. reproductively mature) as a swimming larval form.

This elegant theory, however, is rejected by recent molecular phylogenies of tunicates that suggest their developmental characters are unique and did not give rise to the vertebrates. When Garstang proposed his idea, most researchers accepted that Urochordata branched before Cephalochordata and so the biphasic life cycle was seen as the original state in Chordata. However, because Urochordata are sister clades to Vertebrata, the cephalochordate-vertebrate model is more general, and urochordates more probably evolved their sessile adult phase subsequently (Lowe 2021). In addition, the evidence of developmental studies speaks against the larval origins hypothesis: genomic signalling evidence from hemichordates, cephalochordates, and urochordates shows links among the adult stages, not between larval and adult stages, and a major shift of those signalling sequences would be needed (Lowe 2021).

Studies of animal development are important here, just as they were in identifying the fundamental split among animals between protostomes and deuterostomes (see Figure 1.6). The **development** of the embryos of living vertebrates and other chordates can indicate a great deal about their ancestry. Traditionally, embryos were sliced thinly on a microtome, rather like a mini salami-slicer, and three-dimensional reconstructions were made from scans of the thin sections. Today, these methods are still used, as well as tissue staining and clearing, and CT scanning in which X-rays of small specimens like embryos can be reconstructed to make digital 3D images (see Section 2.1.3). In addition, and most importantly, studies of the **genome** allow developmental biologists to relate specific anatomical structures to genes. In many cases, they have found that genes that code for particular organs or functions are shared among widely different species that may have had enormously long independent histories. So, hypotheses of **homology** between organs can be tested by identifying shared genes and shared elements of the **gene regulatory networks (GRN)**, the collections of molecular regulators that interact with each other and other cellular components to regulate gene expression levels of mRNA and proteins, which together determine the function of the cell. Molecular developmental biologists map the GRNs that specify particular outcomes in embryonic development and these show patterns of homology in function between closely and distantly related organisms.

The interface between such molecular evolutionary studies of development, especially the identification of GRN and their regulation of embryonic developmental patterns, has been called **evo-devo**, short for evolutionary development.

1.5.2 Vertebrates and the Neural Crest

Modern vertebrates are generally defined by two very evident features, the skeleton and the head. The vertebrate internal skeleton can be constructed from cartilage, but in early forms showed at least some mineralized components such as teeth, made from calcium phosphate. The head might seem an odd novelty for vertebrates; after all, any worm or worm-like animal has a head and a tail. However, the vertebrate head has key features, notably a brain and paired sensory organs such as eyes, nostrils, and ears. These two characteristics are referred to by the names of the clade: Vertebrata, the term we use here, refers to their skeleton, notably the vertebrae comprising the backbone, or Craniata, referring to their specialized head features (the **cranium,** the skull).

More generally, vertebrates share many unique characteristics, including the neural crest, the brain, an optic tectum in the brain, paired olfactory capsules, at least one set of semicircular canals, arcualia (embryonic precursors of vertebrae), and closely spaced unpaired fin ray supports (see Box 3.1). A key feature behind many of the unique vertebrate characteristics is the **neural crest**, which is composed of a short-lived cell population that originates in the dorsal neural tube and then migrates all over the body (Ji et al. 2019; Méndez-Maldonado et al. 2020; Rothstein and Simoes-Costa 2023).

The neural crest arises during the gastrulation phase of the vertebrate embryo (see Figure 1.6) when neural crest cells are induced at the edges of the neural plate. During the process of neurulation (Figure 1.11a), the neural plate begins to fold as the edges converge along structures called neural folds, forming the neural tube along the dorsal midline of the developing embryo. The epidermis, an outer

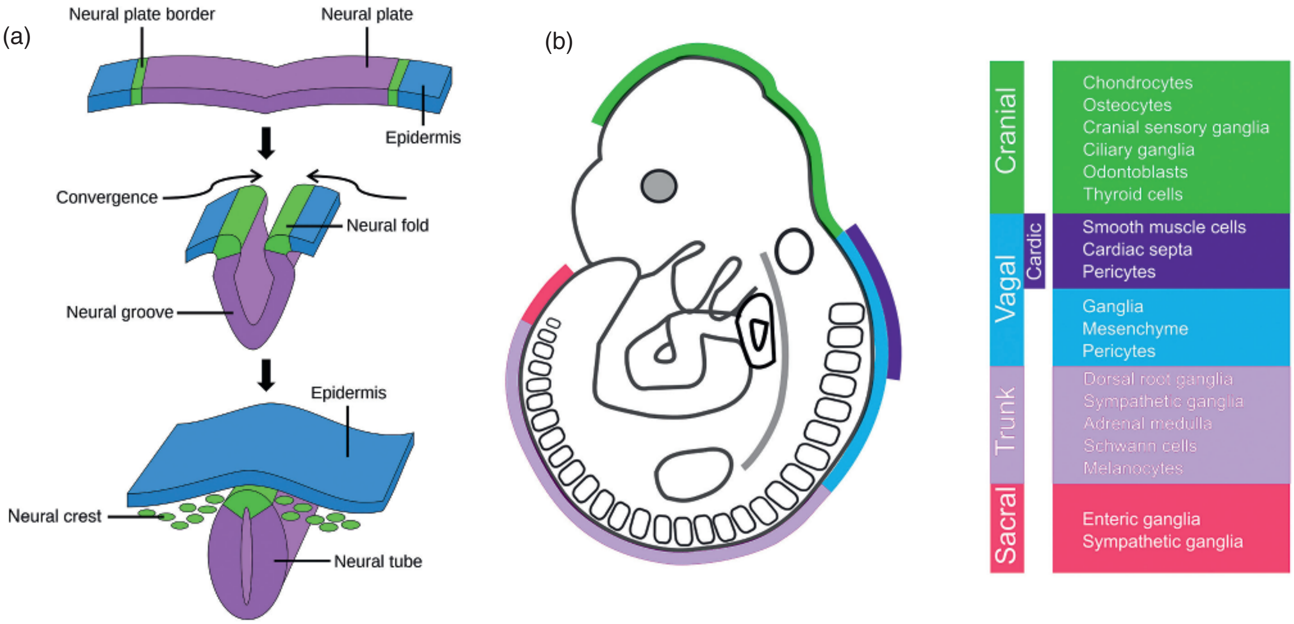


Figure 1.11 Fundamental importance of the neural crest in vertebrate development: (a) formation of neural crest during the process of neurulation, showing, from top to bottom, induction of neural crest cells in the neural plate, folding of the neural groove, and formation of the neural tube and neural crest cell accumulation prior to migration; (b) distinct neural crest cell populations and their major derivative cell types, all colour coded. *Source:* (a) NikNaks/Wikimedia/Licensed under CC BY 4.0. (b) Ji et al. (2019)/MDPI/licensed under CC BY 4.0.

skin layer, extends over the neural tube, and as the embryo continues to develop, neural crest cells migrate away from the neural tube throughout the body. They do this through a process of separation of tissue types into different populations, called delamination.

The neural crest cell populations differentiate according to their position along the anterior-posterior axis of the embryo, into four main functional domains, the cranial neural crest, the vagal and cardiac neural crest, the trunk neural crest, and the sacral neural crest (Figure 1.11b). Neural crest cells from these four regions then migrate throughout the developing embryo and form the basis of 40 or more specialized cell types and components of vertebrate anatomy. For example, the cranial neural crest is the source of the skin, brain, cranial nerves, eye, nose, dental tissues, and jaw and throat bones. The cardiac and vagal neural crest produce the tissues of the heart, and nerve cells around the lungs and digestive system. The trunk neural crest produces bone marrow cells as well as contributing to multiple kinds of nerve cells, chondrocytes (forming cartilage), and hair cells in mammals. The sacral neural crest provides nerve cells, and all regions provide pigment-containing cells in the skin (e.g. melanocytes).

This role of the neural crest in generating most of the key features of Vertebrata has elevated its importance substantially. How did something so fundamental in early embryonic development emerge? Can we see precursors in the other chordates? One point is that the neural plate, the precursor of the neural tube in vertebrates, occurs in the embryos of all bilaterians: this forms as a thickening of the embryonic ectodermal cells, and the borders push up as the neural folds on either side to forms an elongate neural tube, precursor of the brain and spinal cord. All aspects of this process are guided by particular developmental genes shared among all bilaterians (Zhao et al. 2019; York and McCauley 2020).

Importantly, neural crest-like cells have been identified in Urochordata, but they are absent in Cephalochordata, one of the key reasons tunicates are seen as more closely related to vertebrates than amphioxus. The urochordate migratory neural crest-like cells are precursors of pigment, as they are in vertebrates, but these cell populations occupy only a limited area of the developing neural plate border, unlike in vertebrates (York and McCauley 2020). In other tunicate species, similar neural crest-like cells migrate a short distance in the cranial region and differentiate into specialized cells in the ear and eye regions. Others develop in the trunk region and become nerve cells. In all cases, these neural crest-like cells of urochordates are triggered by genomic signalling pathways identical to those in vertebrates. None of these features have been identified in amphioxus.

The neural crest had a special impact on the head region and this was the basis for a remarkable proposal dating back to 1983, called the 'new head' hypothesis (Box 1.4). Perhaps this is the key to understanding how vertebrates evolved.

1.5.3 The Vertebrate Ancestor

In their review of the evidence about the ancestry of vertebrates, Nanglu et al. (2023a) broadly reject the 'bag' hypothesis. They stress that when Urochordata were identified as sister group of Vertebrata and Cephalochordata moved down the phylogenetic tree, the worm-like ancestor was vindicated. This says that the biphasic system in Urochordata, with swimming larva with notochord and adult sessile bag, is secondary, a specialization of the urochordates. The fossils, as we have seen, tend to agree, with sessile forms associated with Urochordata and some Hemichordata, but always easier to interpret as specializations of those evolutionary branches, not ancestral states.

The vermiform hypothesis then prevails. Our chordate ancestor was a wiggling wormy creature, the size and shape of one joint of your little finger. It had no head and swam into food, which it absorbed through the inner linings of its mouth.

Box 1.4 Neural Crest and the Vertebrate Head

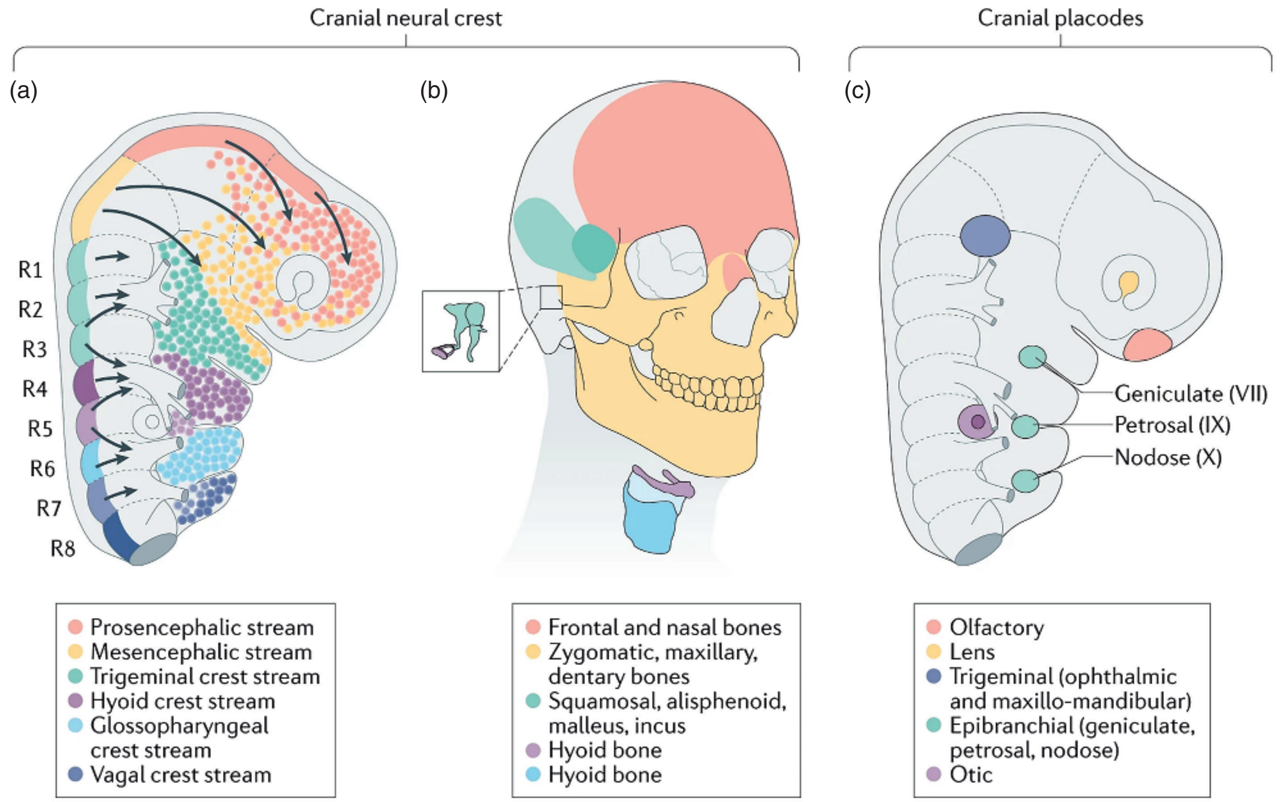
When Carl Gans and Glenn Northcutt proposed the 'new head' hypothesis in 1983 (Gans and Northcutt 1983), they were drawing together their knowledge of vertebrate anatomy and the new evo-devo information about the neural crest, its genomic regulation, and the extraordinary impact of that portion of the early embryo on later development. In particular, they noted how multiple streams of neural crest cells were responsible for every aspect of the head skeleton, the jaws and teeth, the brain, the sensory organs, and the cranial nerves.

Carl Gans (1923–2009) was at the time a professor at the University of Michigan, noted especially for his work on reptile anatomy and evolution, and for editing the 23-volume 'Biology of the Reptilia' series. Glenn Northcutt (born 1941) is a neuroscientist, also then at the University of Michigan, noted for his work on the evolution of the brain in vertebrates.

They envisaged the first chordate as a filter-feeding free swimmer that used its mouth area for breathing through gills and for taking in plankton-sized food. The first step to becoming a vertebrate was that this filter-feeding mechanism evolved to enable selective predation on larger prey items. This diversification of the diet gave the protovertebrates ecological advantages and was also associated with selection for larger overall size (larger predators can eat a wider array of food items). Muscular pumping of the pharynx was required to increase the uptake of oxygen and pharyngeal bars made from collagen were replaced by cartilage bars (as in *Yunnanozoon*) to improve pumping efficiency. The heart and improved circulatory system as well as a muscularized gut all evolved at this time to cope with larger body size and larger prey. Sensory systems (eyes, ears, noses, and electroreception) improved to locate prey, and the brain was also enlarged to handle the new messages. Eventually, a neurocranium evolved together with calcium phosphate mineralization of bones and teeth to enable feeding and protect the brain and sense organs. It's all about the head, and Gans and Northcutt (1983) characterized their idea as a new head added onto the front of the protochordate body rather than being modified from a pre-existing structure.

These ideas are well supported by a modern understanding of the neural crest and its genomic regulation (Martik and Bronner 2021). During early development, cranial neural crest cells migrate from the neural tube to occupy all regions of the embryonic head (illustration a). Ten zones from anterior to posterior can be identified, two within the developing head itself, and eight in the following segments. These regionalized neural crest cells move to pre-specified areas and give rise to particular tissues that may become key bones in the skull and throat, including even the tiny inner ear ossicles (illustration b). At the same time, cranial placodes form, regions of ectodermal tissue that are precursors of individual cranial nerves (illustration c).

All of this is controlled by a pan-neural crest GRN that triggers a sequence of cellular transformations, from specification to epithelial-to-mesenchymal transition, to migration, and ultimately to differentiation (Martik and Bronner 2021). The GRN can be visualized as a series of developmental modules that comprise transcription factors and signalling molecules that interact to drive each of the steps from neural crest cell formation, through migration, to formation of specialized tissues. Initiation of the whole process begins during gastrulation when signalling occurs through the WNT, FGF, and BMP pathways. Elements of these same GRN pathways are seen especially in urochordates, where they specify pigmented cells of the ear (otolith), eye (ocellus), and bipolar tail neurons. This could be the origin of the neural crest and its GRN.



The 'new head' hypothesis is based on studies of anatomy and embryology of modern vertebrates and invertebrate chordates, as well as evo-devo analysis of GRN signalling pathways. (a) During embryonic development neural crest cells migrate (arrows) from ten distinct zones along the neural tube to populate the developing head. (b) The craniofacial skeleton of humans, but also of all vertebrates, is specified by different neural crest migratory cells, as indicated by the colour coding. (c) Cranial placodes form in the early embryo from neural crest precursor cell populations and these then become individual cranial nerves and other structures. *Source:* Adapted from Martik and Bronner (2021). Reproduced with permission.

1.6 Further Reading

A good account of the science covered in this chapter is given by Gee (2018). You can find out more about modern invertebrates, and in particular, those classified as deuterostomes in Barnes et al. (2001), Brusca et al. (2022), and a strongly phylogenetic approach is presented by Waegele et al. (2014), Giribet and Edgecombe (2020), and Schierwater and DeSalle (2021). The embryology and anatomy of modern vertebrates are covered by many zoology texts, such as the classics by Romer and Parsons (1986), Hildebrand and Goslow (2001), and Liem et al. (2001), as well as the more current Kardong (2018) and Pough et al. (2022).

Questions for Future Research

1. What are the closest relatives of chordates among other animal groups?
2. When did the first chordates and the first vertebrates arise?
3. How can new imaging technologies improve our understanding of soft-tissue characters in Cambrian deuterostome fossils?
4. How do the anatomy and physiology of living deuterostomes inform us about early deuterostome and chordate adaptations?
5. Can different phylogenomic analyses be rationalized, for example, to understand why different phylogenetic conclusions may emerge from studies of whole mitochondrial genomes, collections of nuclear genes, and micro-RNAs?

References

Barnes, R., Calow, P., Olive, P.J.W., Golding, G.W. and Spicer, J.I. (2001) *The Invertebrates: a Synthesis*, 3rd edn. Wiley, Oxford.

Bertrand, S. and Escriva, H. (2011) Evolutionary crossroads in developmental biology: amphioxus. *Development* 138: 4819–4830.

Brusca, R.C., Giribet, G. and Moore, W. (2022) *Invertebrates*, 4th edn. Oxford University Press, New York.

Campos-Dávila, L., Pérez-Estrada, C.J., Rodríguez-Estrella, R., Morales-Bojórquez, E., Brun-Murillo, F.G. and Balart, E.F. (2019) Seagrass *Halodule wrightii* as a new habitat for the amphioxus *Branchiostoma californiense* (Cephalochordata, Branchiostomidae) in the southern Gulf of California, Mexico. *ZooKeys* 873: 113–131.

Cannon, J.T., Swalla, B.J. and Halanych, K.M. (2013) Hemichordate molecular phylogeny reveals a novel cold-water clade of harrimaniid acorn worms. *Biological Bulletin* 225: 194–204.

Caron, J.B., Conway Morris, S. and Shu, D. (2010) Tentaculate fossils from the Cambrian of Canada (British Columbia) and China (Yunnan) interpreted as primitive deuterostomes. *PLoS One* 5: e9586.

Caron, J.-B., Morris, S.C. and Cameron, C.B. (2013) Tubicolous enteropneusts from the Cambrian period. *Nature* 495: 503–506.

Chen, J.Y. (2008) Early crest animals and the insight they provide into the evolutionary origin of craniates. *Genesis* 46: 623–639.

Chen J.Y., Huang S.Y., Peng Q.P., Chi H.M., Wang X.Q. and Feng M. (2003) The first tunicate from the Early Cambrian of South China. *Proceedings of the National Academy of Sciences, USA* 100: 8314–8318.

Cong, P.Y., Hou, X.G., Aldridge, R.J., Purnell, M.A. and Li, Y.Z. (2015) New data on the palaeobiology of the enigmatic yunnanozoans from the Chengjiang Biota, Lower Cambrian, China. *Palaeontology* 58: 45–70.

Conway Morris, S. and Caron, J.-B. (2012) *Pikaia gracilens* Walcott, a stem-group chordate from the Middle Cambrian of British Columbia. *Biological Reviews* 87: 480–512.

d'Aniello, S., Bertrand, S. and Escriva, H. (2023) Amphioxus as a model to study the evolution of development in chordates. *eLife* 12: e87028.

Donoghue, P.C.J. and Purnell, M.A. (2009) Distinguishing heat from light in debate over controversial fossils. *BioEssays* 31: 178–189.

Dunn, C.W., Giribet, G., Edgecombe, G.D. and Hejnol, A. (2014) Animal phylogeny and its evolutionary implications. *Annual Review of Ecology, Evolution, and Systematics* 45: 371–395.

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

Gans, C. and Northcutt, R.G. 1983. Neural crest and the origin of vertebrates: a new head. *Science* 220: 268–273.

Garstang, W. (1928) The morphology of Tunicata and its bearing on the phylogeny of the Chordata. *Quarterly Journal of the Microscopical Society* 72: 51–187.

Gee, H. (2018) *Across the Bridge: Understanding the Origin of the Vertebrates*. University of Chicago Press, Chicago.

Giribet, G. and Edgecombe, G.D. (2020) *The Invertebrate Tree of Life*. Princeton University Press, Princeton, NJ.

Hildebrand, M. and Goslow, G. (2001) *Analysis of Vertebrate Structure*. Wiley, New York.

Holland, L.Z. (2015) Cephalochordata. *Evolutionary Developmental Biology of Invertebrates* 6: 91–133.

Hou, X.G., Siveter, D.J., Siveter, D.J., Aldridge, R.J., Cong, P.Y., Gabbott, S.E., Ma, X.Y., Purnell, M.A. and Williams, M. (2017) *The Cambrian Fossils of Chengjiang, China: The Flowering of Early Animal Life*, 2nd edn. Wiley, New York.

Jefferies, R.P.S. (1986) *The Ancestry of the Vertebrates*. British Museum (Natural History), London.

Ji, Y., Hao, H., Reynolds, K., McMahon, M. and Zhou, C.J. (2019) Wnt signaling in neural crest ontogenesis and oncogenesis. *Cells* 8: 1173.

Juravel, K., Porras, L., Höhna, S., Pisani, D. and Wörheide, G. (2023) Exploring genome gene content and morphological analysis to test recalcitrant nodes in the animal phylogeny. *Plos One* 18: e0282444.

Kapli, P., Natsidis, P., Leite, D.J., Fursman, M., Jeffrie, N., Rahman, I.A., Philippe, H., Copley, R.R. and Telford, M.J. (2021) Lack of support for Deuterostomia prompts reinterpretation of the first Bilateria. *Science Advances* 7: eabe2741.

Kardong, K.V. (2018) *Vertebrates: Comparative Anatomy, Function, Evolution*, 8th edn. McGraw-Hill, New York.

Kaul-Strehlow, S. and Röttinger, E., 2015. Hemichordata. *Evolutionary Developmental Biology of Invertebrates* 6: 59–89.

Laumer, C.E., Fernández, R., Lemer, S., Combosch, D., Kocot, K.M., Riesgo, A., Andrade, S.C., Sterrer, W., Sørensen, M.V. and Giribet, G. (2019) Revisiting metazoan phylogeny with genomic sampling of all phyla. *Proceedings of the Royal Society B* 286: 20190831.

Li, Y., Kocot, K.M., Tassia, M.G., Cannon, J.T., Bernt, M. and Halanych, K.M. (2019) Mitogenomics reveals a novel genetic code in Hemichordata. *Genome Biology and Evolution* 11: 29–40.

Li, Y., Dunn, F.S., Murdock, D.J., Guo, J., Rahman, I.A. and Cong, P. (2023) Cambrian stem-group ambulacrarians and the nature of the ancestral deuterostome. *Current Biology* 33: 2359–2366.

Liem, K., Bemis, W., Walker, W.F., Jr. and Grande, L. (2001) *Functional Anatomy of the Vertebrates: An Evolutionary Perspective*, 3rd edn. Thomson Brooks/Cole, Philadelphia.

Lowe, C.J. (2021) Molecular insights into deuterostome evolution from hemichordate developmental biology. *Current Topics in Developmental Biology* 141: 75–117.

Maletz, J. (2019) Tracing the evolutionary origins of the Hemichordata (Enteropneusta and Pterobranchia). *Palaeoworld* 28: 58–72.

Marletaz, F., Peijnenburg, K., Goto, T., Satoh, N. and Rokhsar, D.S. (2019) A new spiralian phylogeny places the enigmatic arrow worms among gnathiferans. *Current Biology* 29: 312–318 e313.

Martik, M.L. and Bronner, M.E. 2021. Riding the crest to get a head: neural crest evolution in vertebrates. *Nature Reviews Neuroscience* 22: 616–626.

McGrath, C. (2022) Highlight: unraveling the origins of LUCA and LECA on the Tree of Life. *Genome Biology and Evolution* 14: evac072.

Méndez-Maldonado, K., Vega-López, G.A., Aybar, M.J. and Velasco, I. (2020) Neurogenesis from neural crest cells: molecular mechanisms in the formation of cranial nerves and ganglia. *Frontiers in Cell and Developmental Biology* 8: 635.

Nanglu, K., Caron, J.B. and Cameron, C.B. (2020) Cambrian tentaculate worms and the origin of the hemichordate body plan. *Current Biology* 30: 4238–4244.

Nanglu, K., Cole, S.R., Wright, D.F. and Souto, C. (2023a) Worms and gills, plates and spines: the evolutionary origins and incredible disparity of deuterostomes revealed by fossils, genes, and development. *Biological Reviews* 98: 316–351.

Nanglu, K., Lerosey-Aubril, R., Weaver, J.C. and Ortega-Hernández, J. (2023b) A mid-Cambrian tunicate and the deep origin of the ascidiacean body plan. *Nature Communications* 14: 3832.

Ou, Q.A., Morris, S.C., Han, J., Zhang, Z.F., Liu, J.N., Chen, A.L., Zhang, X.L. and Shu, D.G. (2012) Evidence for gill slits and a pharynx in Cambrian vetulicolians: implications for the early evolution of deuterostomes. *BMC Biology* 10: 81.

Parry, L.A., Smithwick, F., Nordén, K.K., Saitta, E.T., Lozano-Fernandez, J., Tanner, A.R., Caron, J.B., Edgecombe, G.D., Briggs, D.E. and Vinther, J. (2018) Soft-bodied fossils are not simply rotten carcasses–toward a holistic understanding of exceptional fossil preservation: exceptional fossil preservation is complex and involves the interplay of numerous biological and geological processes. *BioEssays* 40: 1700167.

Peterson, K.J. and Eernisse, D.J. (2016) The phylogeny, evolutionary developmental biology, and paleobiology of the Deuterostomia: 25 years of new techniques, new discoveries, and new ideas. *Organisms Diversity & Evolution* 16: 401–418.

Peterson, K.J., Su, Y.H., Arnone, M.I., Swalla, B. and Kiong, B.L. (2013) MicroRNAs support the monophyly of enteropneust hemichordates. *Journal of Experimental Zoology, B Molecular and Developmental Evolution* 320: 368–374.

Pough, F.H., Bemis, W.E., McGuire, B.A. and Janis, C.M. (2022) *Vertebrate Life*, 11th edn. Oxford University Press, New York.

Romer, A.S. and Parsons, T.S. (1986) *The Vertebrate Body*, 6th edn. W.B. Saunders, Philadelphia.

Rothstein, M. and Simoes-Costa, M. (2023) On the evolutionary origins and regionalization of the neural crest. *Seminars in Cell & Developmental Biology* 138: 28–35.

Röttinger, E. and Lowe, C.J. 2012. Evolutionary crossroads in developmental biology: hemichordates. *Development* 139, 2463–2475.

Saleh, F., Qi, C., Buatois, L.A., Mángano, M.G., Paz, M., Vaucher, R., Zheng, Q., Hou, X.G., Gabbott, S.E. and Ma, X. (2022) The Chengjiang Biota inhabited a deltaic environment. *Nature Communications* 13: 1569.

Sansom, R.S., Gabbott, S.E. and Purnell, M.A. (2010) Non-random decay of chordate characters causes bias in fossil interpretation. *Nature* 463: 797–800.

Schierwater, B. and DeSalle, R. (2021) *Invertebrate Zoology: A Tree of Life Approach*. CRC Press, Baton Rouge, LA.

Shu, D.G., Morris, S.C. and Zhang, X.L. (1996) A *Pikaia*-like chordate from the Lower Cambrian of China. *Nature* 384: 157–158.

Shu, D.G., Conway Morris, S., Zhang, Z.F. and Han, J. (2010) The earliest history of the deuterostomes: the importance of the Chengjiang Fossil-Lagerstatte. *Proceedings of the Royal Society B* 277: 165–174.

Stach, T. and Kaul, S. (2011) The postanal tail of the enteropneust *Saccoglossus kowalevskii* is a ciliary creeping organ without distinct similarities to the chordate tail. *Acta Zoologica* 92: 150–160.

Swalla, B.J. and Smith, A.B. (2008) Deciphering deuterostome phylogeny: molecular, morphological, and palaeontological perspectives. *Philosophical Transactions of the Royal Society B* 363: 1557–1568.

Tassia, M.G., Cannon, J.T., Konikoff, C.E., Shenkar, N., Halanych, K.M. and Swalla, B.J. (2016) The global diversity of Hemichordata. *PLoS One* 11: e0162564.

Telford, M.J., Budd, G.E. and Philippe, H. (2015) Phylogenomic insights into animal evolution. *Current Biology* 25: R876–R887.

Tian, Q., Zhao, F., Zeng, H., Zhu, M. and Jiang, B. (2022) Ultrastructure reveals ancestral vertebrate pharyngeal skeleton in yunnanozoans. *Science* 377: 218–222.

Waegele, J.W., Bartholomaeus, T.W. and Misof, B. (eds) (2014) *Deep Metazoan Phylogeny: The Backbone of the Tree of Life: New Insights from Analyses of Molecules, Morphology, and Theory of Data Analysis*. Walter de Gruyter, Berlin.

Williams, T.A., Cox, C.J., Foster, P.G., Szöllősi, G.J. and Embley, T.M. (2020) Phylogenomics provides robust support for a two-domains tree of life. *Nature Ecology & Evolution* 4: 138–147.

York, J.R. and McCauley, D.W. (2020) The origin and evolution of vertebrate neural crest cells. *Open Biology* 10: 190285

Zamora, S., Lefebvre, B., Javier Alvaro, J., Clausen, S., Elicki, O., Fatka, O., Jell, P., Kouchinsky, A., Lin, J.P., Nardin, E. and Parsley, R. (2013) Chapter 13 Cambrian echinoderm diversity and palaeobiogeography. *Geological Society, London, Memoirs* 38: 157–171.

Zhang, Q.L., Zhang, G.L., Yuan, M.L., Dong, Z.X., Li, H.W., Guo, J., Wang, F., Deng, X.Y., Chen, J.Y. and Lin, L.B. (2018) A phylogenomic framework and divergence history of Cephalochordata amphioxus. *Frontiers in Physiology* 9: 1833.

Zhao, F.C., Caron, J.-B., Bottjer, D.J., Hu, S.X., Yin, Z.J. and Zhu, M.Y. (2013) Diversity and species abundance patterns of the Early Cambrian (Series 2, Stage 3) Chengjiang Biota from China. *Paleobiology* 40: 50–69.

Zhao, D., Chen, S. and Liu, X. (2019) Lateral neural borders as precursors of peripheral nervous systems: a comparative view across bilaterians. *Development, Growth & Differentiation* 61: 58–72.