nature INSIGHT

ORIGIN AND EVOLUTION OF VERTEBRATES

23 April 2015 / Vol 520 / Issue No 7548

Cover illustration
Jasiek Krzysztofiak

The Macmillan Building
4 Crinan Street
London N1 9XW, UK
Tel: +44 (0) 20 7833 4000
e: nature@nature.com

nature publishing group

To celebrate the golden jubilee of *On the Origin of Species*, in 1909, the Linnean Society of London held a special meeting on a hot biological topic of the day — the origin of the vertebrates. Such was the lack of consensus that one commentator, the zoologist T. R. R. Stebbing, wrote that "the disputants agreed on one single point, namely, that their opponents were all in the wrong."

The problem is easily stated — vertebrates have so many special features, from large brains to complex physiologies to unique tissues such as enamel and bone — that their evolution from invertebrates is obscure. The question had intrigued Aristotle, and foxed minds as keen as those of William Bateson and Thomas Hunt Morgan, who, by way of finding a more rewarding problem, went off to discover genetics instead.

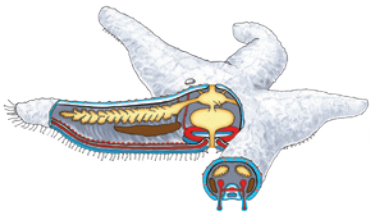
The same tools that Bateson and Hunt Morgan helped to create have now returned to address the old problem. Although our understanding is far from complete, it is much better than it was even 20 years ago, and is summarized in this collection of reviews.

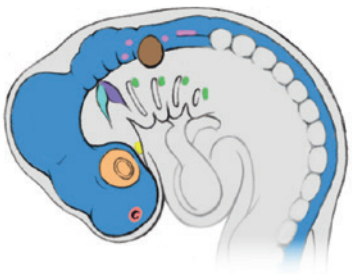
Nicholas Holland and colleagues set out how the varied theories advanced to explain vertebrate origins, before Lowe *et al.* show how they fit in to the deuterostomes, a larger branch of the animal kingdom. Diogo *et al.* add new perspectives to a central question of vertebrate origins, namely, the origin of the head. Marianne Bronner and colleagues then look at the embryonic tissue known as neural crest, another uniquely vertebrate feature. Philippe Janvier surveys the wealth of newly found, and often curious, fossil evidence, and Martin Brazeau and Matt Friedman chart the evolution of jawed vertebrates from jawless forms. If Stebbing was able to peruse this collection, I hope he would agree that we have come a long way.

Henry Gee
Senior Editor

CONTENTS

REVIEWS





REVIEW

doi:10.1038/nature14433

Scenarios for the making of vertebrates

Nicholas D. Holland[1], Linda Z. Holland[1] & Peter W. H. Holland[2]

Over the past 200 years, almost every invertebrate phylum has been proposed as a starting point for evolving vertebrates. Most of these scenarios are outdated, but several are still seriously considered. The short-range transition from ancestral invertebrate chordates (similar to amphioxus and tunicates) to vertebrates is well accepted. However, longer-range transitions leading up to the invertebrate chordates themselves are more controversial. Opinion is divided between the annelid and the enteropneust scenarios, predicting, respectively, a complex or a simple ancestor for bilaterian animals. Deciding between these ideas will be facilitated by further comparative studies of multicellular animals, including enigmatic taxa such as xenacoelomorphs.

Biologists have considered nearly every major taxon of animals as the key starting point for the evolution of vertebrates. We survey these ideas, many of which are no longer tenable in the light of subsequent advances in biology, and then concentrate on the few scenarios that are currently the subject of major research programmes. Lamarck was the first to propose an evolutionary conversion from an invertebrate to a vertebrate. In 1809, he depicted a phylogenetic tree, including an invertebrate-to-vertebrate transition in which molluscs gave rise to fishes[1]. During the next few decades, several others speculated on how body plans of invertebrates and vertebrates might be related; however, those biologists were generally in search of an underlying unity of organismal design. Evolution was not explicitly mentioned by key figures such as Geoffroy Saint-Hilaire[2], although one senses that he was on the verge of believing in it.

Aside from Lamarck's proposal, explicitly evolutionary schemes that derived vertebrates from invertebrates started appearing only after the publication of *On the Origin of Species* in 1859. In general, the scenarios were based on the morphology of developmental stages and adults of extant animals. Palaeontological evidence was considered less often[3–5], and molecular evidence was not widely considered until the 1980s with the advent of molecular phylogenetics and evolutionary developmental biology.

In Fig. 1, scenarios for the origin of vertebrates are arranged on a timeline extending from the publication of *On the Origin of Species* to the present. The references are broadly divided into those focused on larval type and those concerned with adults — a dichotomy reflecting two opposing views of life-history evolution. The first considers pelagic larvae as primal with benthic stages added later, and the second considers benthic stages as primitive with pelagic larvae interpolated later. Classification of the scenarios is not straightforward because relatively few proposed a linear ancestor–descendant relationship. More commonly, they were presented in the context of branching, sister-group relationships. For example, if enteropneusts were considered the sister group of the chordates (as in Fig. 2a), the ancestral node is often referred to as enteropneust-like. Finally, when a given scheme involves an evolutionary pathway through several major taxa to the vertebrates, the scenario is named for the invertebrate group receiving the most attention from the original author.

Scenarios currently the subject of active research

Contemporary research on the origin of vertebrates from invertebrates falls into two broad categories: the short-range transition from invertebrate chordates (amphioxus-like and tunicate-like ancestors) to vertebrates, and longer-range transitions from the base of bilaterally symmetrical animals or from the base of deuterostomes to vertebrates. Scenarios starting with invertebrate chordates are less controversial than the two long-range scenarios being actively studied: the annelid and the enteropneust theory.

Invertebrate chordate to vertebrate transition

The nearest relatives of the vertebrates are the invertebrate chordates, although it is still not settled whether chordate evolution should be considered from the viewpoint of larvae being primal[6] or larvae being interpolations[7,8]. Although invertebrate chordate scenarios ignore the deeper history of the vertebrate lineage, they still centre on events initiated more than 500 million years ago and involve remarkable evolutionary changes that are considered in companion reviews in this issue. Recently, the major chordate taxa were rearranged (Fig. 2b) on the basis of morphology and molecular phylogenetics, which have decisively shown that amphioxus is the sister group to tunicates and vertebrates[9,10]. The new arrangement implies that the tunicates have secondarily lost segmentation, coeloms and kidneys, but are vertebrate-like in features such as intercellular tight junctions, proto-neural crest, striated heart muscles, proto-placode derivatives and voluminous blood plasma with abundant circulating corpuscles.

The annelid theory

The first of the two long-range scenarios is the annelid theory. When initially published 140 years ago, it proposed a direct conversion of annelid worms into vertebrates[11,12]. Now, however, the starting point is often considered to be an annelid-like urbilaterian[13] (Fig. 2a, b). The annelid theory has its roots in arthropod biology, because these two groups were long considered to be very close relatives (Fig. 2a), and results for one were generally considered to be valid for the other.

In the original annelid scenario, Dohrn[11] started with a worm that inverted the body on the way to evolving into a vertebrate, thus positioning the old mouth on the top of the head and necessitating the formation of a new mouth on the ventral side of the body; thereafter, the old mouth disappeared, while the new one persisted (Fig. 3a–c). Several of Dohrn's colleagues modified his scenario in attempts to improve it[14–21], but the theory went into eclipse early in the twentieth century when the bilaterian animals were rearranged into two superphyla — the protostomes and the

[1]Marine Biology Research Division, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093, USA. [2]Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

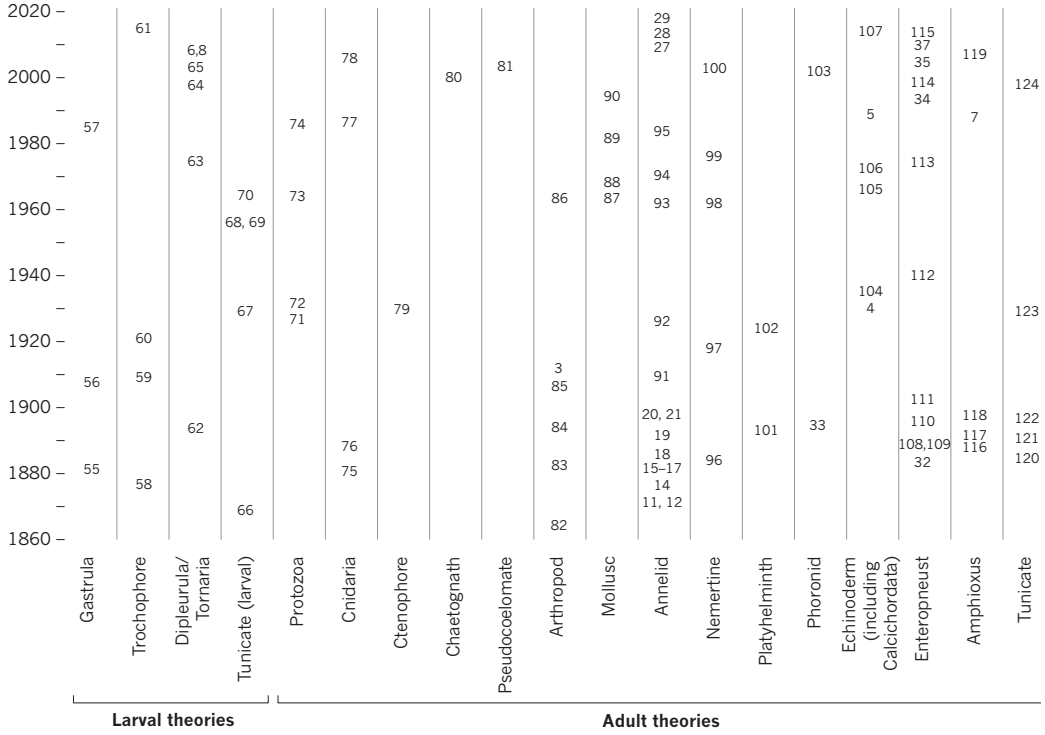


Figure 1 | Scenarios for the invertebrate-to-vertebrate transition. Each scenario is categorized according to the larval type or to the taxon of adult invertebrate proposed as ancestral to the vertebrates[3–8,11,12,14–21,27–29,32–35,37,55–124]. For prolix authors, only their most inclusive publications are given. Also omitted are references (typically textbooks) that repeat previous ideas without adding new information. References to problematic fossil ancestors of vertebrates are not included (except calcichordates, which are considered to be echinoderms here).

deuterostomes. The resulting relocation of annelids and arthropods at a considerable phylogenetic distance from the vertebrates (Fig. 2a) weakened the idea of a complex urbilaterian and shifted opinion towards a simple urbilaterian, which was imagined to be rather like an acoel flatworm that independently gave rise to annelids and vertebrates with their complex, but only superficially similar, body plans.

In the 1990s, advances in developmental genetics — again with arthropods leading the way — set the stage for the revival of the annelid theory. The fly *dpp* gene was found to be expressed dorsally and to have dorsalizing activity, whereas the homologous frog *bmp4* was expressed ventrally and found to have ventralizing activity[22]. Arendt and Nübler-Jung interpreted this pattern as support for homology between arthropod and vertebrate nerve cords and indicative of a dorsoventral inversion of the body during the invertebrate-to-vertebrate transition[23]. The proposed nerve-cord homology was strengthened by the discovery that the fly *sog* gene was expressed ventrally and had ventralizing activity, whereas the homologous frog *chordin* gene was expressed dorsally and had dorsalizing activity. In addition, *sog*/*chordin* and *dpp*/*bmp4* antagonized one another to establish a dorsoventral axis that was reversed between flies and frogs[24]. Additional support came from the finding that neural progenitor cells in the central nervous system (CNS) were organized in longitudinal bands each characterized by a distinctive suite of gene expression that was homologous between flies and vertebrates, and that gene expression in these bands was comparable mediolaterally in both organisms[25].

The developmental genetic comparison between arthropods and vertebrates[22–25], reinforced by details from neurochemistry and neural circuitry, favoured the revival of the inverted annelid theory. Direct comparisons between annelids and vertebrates also revealed commonalities in anterior–posterior regionalization by Hox genes[26], genetic specification of several kinds of nerve cells[27,28] and the formation of notochord-like structures[29]. As already mentioned, the revived annelid scenario posits the evolution of an already complex urbilaterian ancestor into a vertebrate. Such a transition would be most parsimonious if it proceeded through consistently complex intermediates. However, some features, such as segmentation and a clearly centralized nerve cord, are absent from several taxa associated with the presumed evolutionary lineage that leads to the vertebrates, possibly due to secondary losses. Such losses would have occurred in echinoderms, at least some hemichordates and xenacoelomorphs, although the deuterostome nature of the last has not yet been firmly established[30,31]. Continuity between annelid-like ancestors and vertebrates could be strengthened if complex, segmented fossils of basal deuterostomes were known. Although several such fossils have been proposed as ancestral deuterostomes (vetulicolians and *Herpetogaster*), their taxonomic affinities remain highly controversial.

The enteropneust theory

The second long-range scenario of vertebrate origins currently under active study is the enteropneust theory. These marine worms (Fig. 3d), characterized by three body regions (proboscis, collar and trunk), belong to the Hemichordata, a phylum that also includes the minute pterobranchs (Fig. 3e), which comprise a flattened oral shield corresponding to the enteropneust proboscis, a collar extending into tentacle-fringed arms and a trunk. According to the original enteropneust theory[32], proposed by Bateson in 1886, the body axis of enteropneusts was not inverted relative to that of vertebrates. For him, the stomochord (Fig. 3d) corresponded to a vertebrate notochord, the collar cord (which he considered dorsal) corresponded to the vertebrate CNS, and the pharyngeal gill slits in both groups were homologous. Such an enteropneust was much like a vertebrate except that it lacked segmented musculature along the anterior–posterior axis. At the time, Bateson was uncertain about the deeper evolutionary source of the enteropneusts, although he tentatively suggested that they might have evolved from nemerteans or even tunicates. However, at the close of the nineteenth century, Masterman[33] proposed what seemed to be a firmer connection between enteropneusts and the rest of the animal kingdom through relatively complex precursors — pterobranchs (already mentioned) and the worm-like phoronids, which live mostly buried, but extend their tentacle crown into the sea water.

Through much of the twentieth century, Bateson's hypothesis, although not universally accepted, persisted. This inactivity ended in 1996, when Nübler-Jung and Arendt made a striking alteration[34]. They proposed that enteropneusts had an annelid-like CNS comprising three contiguous nerve tracts (the collar cord, the circumenteric nerve ring and the trunk ventral nerve cord), all recognizable by their giant nerve fibres. Such an enteropneust (Fig. 3f) complemented their earlier revival of the annelid theory[23] by approximating an intermediate stage in the conversion of a complex urbilaterian into a vertebrate. Because this CNS was oriented

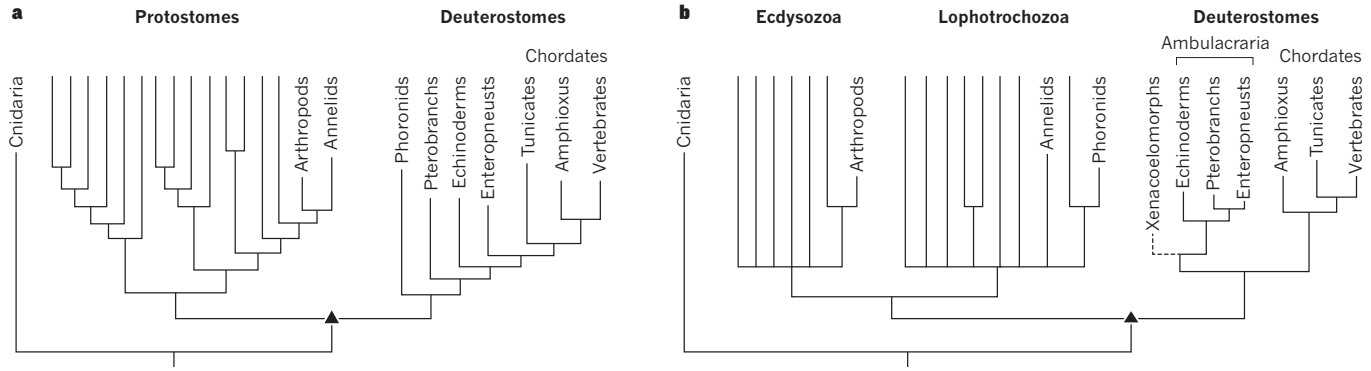


Figure 2 | Simplified trees of metazoan animal life. Taxa not mentioned in this Review are indicated by unlabelled branches (that are reduced in number and intended to be diagrammatic); the position of the Urbilateria is indicated by a triangle. **a**, Morphology-based tree[65]. **b**, Sequence-based tree[125]; the dashed line emphasizes the current uncertainty about the placement of the xenacoelomorphs.

as in annelids, the conversion into a vertebrate-like descendant (Fig. 3g) would require dorsoventral inversion, in contrast to Bateson's original scenario. While Nübler-Jung and Arendt were revising the enteropneust theory[34], molecular phylogenetics revealed that the relatively complex phoronids are neither deuterostomes nor their close relatives[30] (Fig. 2b). One interpretation of the new phylogeny was that the ancestors of the enteropneusts had relatively simple body plans — traceable back to an even simpler urbilaterian. The new phylogenetic arrangement triggered the definitive revival of the enteropneust theory that is still in progress.

The chief proponent of this newest revival of the enteropneust theory is Lowe, who gathered support for it with developmental genetic studies (see Review on page 456). He first considered a score of genes with homologues patterning the vertebrate CNS along its anterior–posterior axis[35]. Most of these enteropneust genes were expressed in the same anterior–posterior order as their homologues in the vertebrate CNS — but in annular bands of ectoderm and not in any tissue that might be interpreted as a CNS[35]. He concluded that the nervous system lacked any CNS component and consisted exclusively of an ectodermal nerve net. Although vertebrate homologues of many of the genes studied by Lowe help to establish borders separating neuronal populations in the vertebrate CNS[36], no corresponding neuroanatomical or neurophysiological discontinuities have yet been found in any enteropneust tissue. In Lowe's original scenario, the transition of enteropneust-like ancestors into vertebrates involved a loss of most of the ectodermal neurons, except along the midline of the body, where a CNS was elaborated. By similar, but independent paths, the dispersed nerve net of a structurally simple urbilaterian would have given rise to the complex CNS of annelids and arthropods.

Lowe subsequently studied the genes involved in establishing the dorsoventral axis of enteropneusts[37] and found that *BMP* and *chordin* were expressed, respectively, on the dorsal and ventral sides — if the body is assumed to be oriented similarly to annelids and arthropods. However, unlike the situation in amphioxus and vertebrates, upregulation experiments failed to alter neuron distribution, although some non-neural structures (the mouth, for example) were repositioned as expected. These results suggested that the *BMP–chordin* axis initially patterned exclusively non-neural structures and only later in evolution became linked to positioning neurons. This linkage to neural development was thought to have occurred independently in annelids, arthropods and vertebrates. Lowe[37] considered, but initially rejected, the converse possibility: that the relation between dorsoventral signalling and nervous-system development was ancient and was secondarily lost in the lineage leading to the enteropneusts.

More recent work challenges one point in the revived enteropneust scenario: that no CNS is present. First, Nomaksteinsky *et al.*[38] suggested that the proboscis plexus, collar cord, circumenteric nerves, and trunk dorsal and ventral cords have some properties of a CNS — cell bodies of neurons are present and extend their neurites into an adjacent neuropil — and that the epidermis outside the nerve cords includes only widely scattered nerve cells representing a sparse peripheral nervous system instead of a nerve net. In addition, Cunningham and Casey[39] found enteropneust neuronal marker genes expressed along both the dorsal and ventral cord of the trunk, which they too suggested might be parts of a CNS. Neither study could resolve the dorsoventral orientation of the enteropneust body. In an attempt to answer this question, the left–right asymmetry of *Nodal* gene expression was compared during development of several deuterostomes. Right-sided expression in echinoderms and enteropneusts contrasted with left-sided expression in vertebrates, indicating that the dorsoventral axis of vertebrates is indeed inverted relative to that of echinoderms and enteropneusts[40,41]. As a caveat, however, although *Nodal* is involved in establishing the left–right axes of echinoderms and vertebrates, it evidently has no comparable functional role in enteropneusts[42].

To complicate matters further, Miyamoto and Wada[43] found that the endoderm of the enteropneust stomochord and the roof of the buccal cavity are sources of Hedgehog signals that evidently induce and pattern the collar nerve cord. This parallels Hedgehog signalling from the notochord to the nascent neural tube during vertebrate development. Their data could be interpreted to mean that dorsoventral inversion did not take place during the enteropneust-to-vertebrate transition, that the stomochord is homologous to a notochord, and that the collar cord corresponds to at least part of the vertebrate CNS. These conclusions are close to those reached by Bateson in his original scenario[32], although Miyamoto and Wada acknowledge that co-option of gene networks cannot be ruled out. These disagreements about the enteropneust nervous system seem likely to be resolved by additional neuroanatomical studies. However, that would still leave the nature of the urbilaterian unsettled, which will be considered in the next section.

Progress, problems and prospects

At the end of an argumentative symposium on the origin of vertebrates a century ago[44], one participant summed up progress with the mischievous words: "When we return home and our friends gleefully enquire, 'What then has been decided as to the Origin of Vertebrates?', so far we seem to have no reply ready, except that the disputants agreed on one single point, namely, that their opponents were all in the wrong." Although prospects for solving the riddle of vertebrate origins at that time did not look good, there has been progress. In particular, we now know where vertebrates fit in the animal phylogenetic tree. This knowledge helps to refine the remaining questions. To start with, we can consider an evolutionary tree as including a nested series of ancestors, each defining a different node of the tree, progressively deeper in time. As we climb down the tree, back in time from the living vertebrates, we encounter each ancestral node in turn. As we proceed, we should not be asking what did the ancestor of vertebrates look like? But instead what did each successive ancestor of the vertebrates look like? This logic can be applied to the node-based ancestors, but we should remember that there must have been an unbroken, genealogically connected series of ancestors between each node that are all but invisible to comparative biology based on living taxa.

Logically, the most recent node-based ancestor of all living vertebrates was itself a vertebrate, and possessed characters shared by lampreys,

hagfish and jawed vertebrates. This animal, living more than half a billion years ago, had a well-developed head and brain, complex cranial sense organs, segmented musculature and a vertebral column (recently shown to be present but secondarily reduced in hagfish[45]), but no jaws or paired fins. The ancestor also probably shared the genome duplications that set vertebrates apart from other deuterostomes. The subsequent course of evolution in the vertebrates is considered in several companion Reviews in this Insight; however, here we are concerned with looking the other way — towards the invertebrate roots of the vertebrates.

The two closest lineages to the vertebrates are the tunicates and the cephalochordates (such as amphioxus). Like vertebrates, both are chordates. The chordate ancestor had segmented muscle blocks, a notochord and a dorsal CNS. It also probably gathered food particles on secretions produced by a glandular endostyle located in an expanded, perforated pharynx. Controversy remains over what the head region of this long-extinct ancestor looked like, because the anterior region of tunicates (or their larvae) is so different from that of amphioxus. Did this ancestor have mesodermal somites (segments) in its anterior region, like a modern amphioxus, or was the anterior unsegmented as it is in tunicate larvae? This may sound like a minor issue, but it is important to resolve if we wish to understand how our own head and brain arose in evolution. This old debate remains unsettled, and more work is needed to compare gene expression and cellular fates in the cranial regions of each chordate group as well as between the cranial and somitic mesoderm of vertebrates.

At the next node-based ancestor, the basal deuterostome, the rival claims of the annelid and enteropneust theories first begin competing for our attention. The chordates are the sister group to the Ambulacraria[46], a clade comprising enteropneusts, pterobranchs and echinoderms (Fig. 2b). Somewhere in the mix may also be the acoels and nemertodermatids (tiny animals with an inconspicuous nervous system) and possibly the larger, but similarly simple, xenoturbellids (here, we will accept the unification of these three groups as xenocoelomorphs[31,47]). The placement of the xenacoelomorphs in the evolutionary tree is also debated; for example, molecular phylogenies that place them as sisters to Ambulacraria — plus or minus the chordates — do not sit easily with other features such as their simple Hox gene cluster[47]. To understand the importance of xenocoelomorphs, we need to consider the common ancestor of Ambulacraria and Chordata. This animal in our series of vertebrate ancestors possessed pharyngeal slits (homologous in enteropneusts and chordates[48]), but what else? Did it have a brain and a CNS, for example? Chordates have a dorsal centralized nerve cord, whereas at least echinoderms have a dispersed nervous system that may be relatively condensed in some regions and not generally considered a CNS, although there is an element of subjectivity in deciding what constitutes a CNS. The putative CNS nature of enteropneust nerve cords[38] has been noted earlier. A similar debate surrounds xenacoelomorphs: xenoturbellids are not considered to have a brain, whereas acoels and nemertodermatids have small anterior aggregations of neural tissue that some have considered to be brain-like[47]. If xenacoelomorphs are basal in the deuterostomes, one might envisage the common ancestor of chordates and ambulacrarians to be enteropneust-like in lacking a clear CNS and a 'brain', although secondary simplification might have occurred[31].

Does this mean that the enteropneust theory wins over the annelid theory? Unfortunately, things are not simple. First, centralized nerve cords are widely distributed (although far from the rule) among bilaterian animals. Thus, concluding that the urbilaterian (and in turn the later ambulacrarian and chordate common ancestor) possessed a CNS would not be

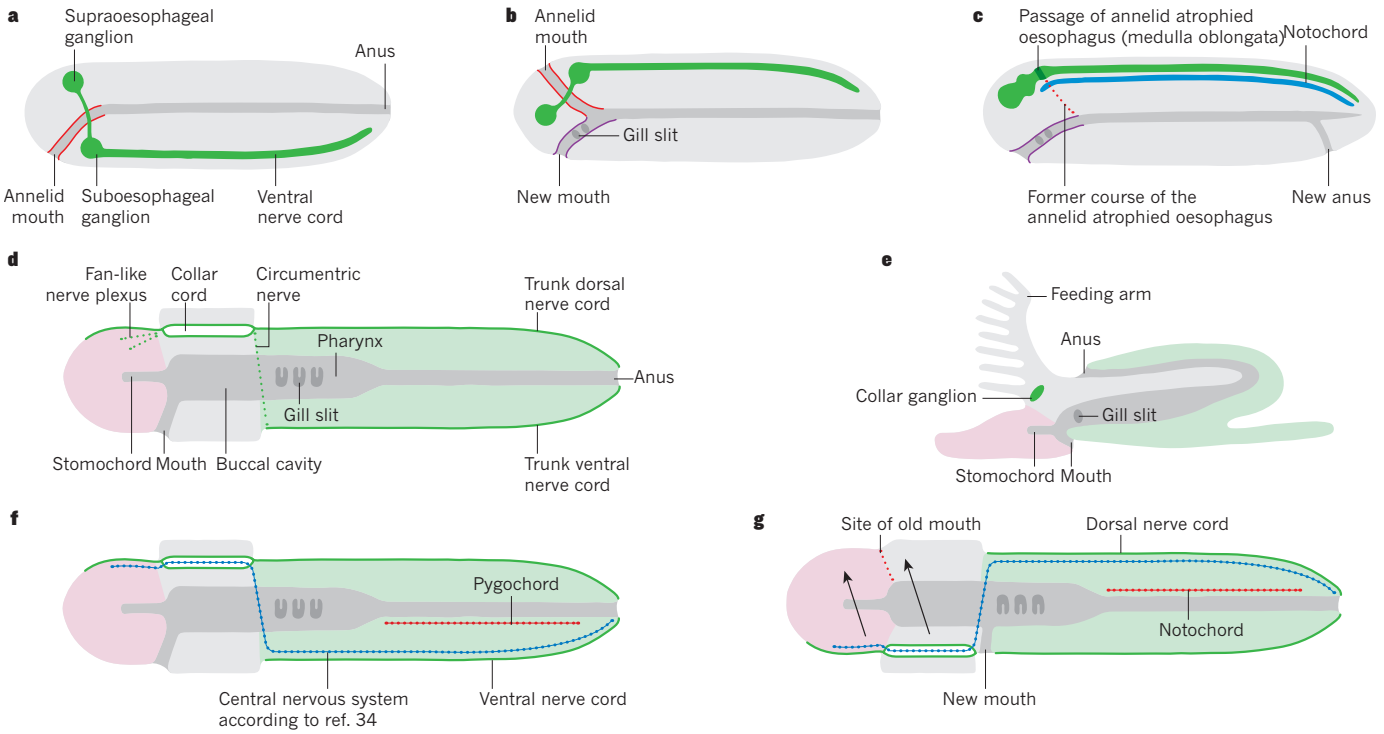


Figure 3 | Annelid and enteropneust theories. **a**, An annelid with a central nervous system (CNS; green) comprising supraoesophageal and suboesophageal ganglia, circumoesophageal connectives and ventral nerve cord. **b**, Dorsoventral inversion[11] produces a new foregut (purple) penetrated by gill slits. **c**, Annelid-to-vertebrate transition. The new foregut persists, but the old one atrophies, permitting union of the supra- and suboesophageal ganglia into a vertebrate-like brain. A notochord (blue) originates from connective tissue surrounding the nerve cord, and a new anus opens. **d**, Enteropneust according to Bateson[32], showing proboscis (pink), collar (grey) and trunk (light green). The ventral mouth opens into a buccal cavity, giving off a small diverticulum (the stomochord) anteriorly and connecting with the pharynx posteriorly. Gill slits penetrate either side of the pharynx, and the post-pharyngeal gut ends posteriorly at the anus. **e**, A pterobranch hemichordate (*Rhabdopleura*), comprising a cephalic shield (pink), collar with feeding arms (grey) and trunk (light green). **f**, Enteropneust as conceived by Nübler-Jung and Arendt[34] with the blue line showing the extent of the CNS. The red line indicates the pygochord. **g**, Proposed inversion during enteropneust-to-vertebrate transition[34]. The pygochord becomes the notochord; the trunk ventral nerve cord becomes the dorsal nerve cord; a dorsal shift of the proboscis plexus and collar cord (arrows) supplies anterior brain regions; and a new mouth forms, while the old one disappears. The transition proposed by Nübler-Jung and Arendt (shown here between **f** and **g**) has now been supplanted by the more current scenario of Lowe[35,37], which is covered in detail by the Review on page 456.

unreasonable. This would imply secondary reduction in basal deuterostomes. A key issue is whether the deuterostome ancestor was segmented along the body axis[49]. This question is inextricably linked to the question of whether the urbilaterian was also segmented. Several distantly related invertebrates are segmented along the body axis, including arthropods and annelids. If their segmentation is homologous with that of chordates, then, as has been suggested[50], the ancestors of both deuterostomes and protostomes were segmented, and enteropneusts lost their segments.

However, the segmentation issue is still vigorously debated. Molecular similarities in the control of segmentation between arthropods and some annelids are striking[6,51], and there are commonalities in gene expression between mesodermal segmentation in these two phyla and in chordates[52]. Even so, deciding whether the similarities in segmentation are due to inheritance from a common ancestor or to independent co-option of parts of the same molecular machinery[53] is not straightforward. New modes of segmentation (in the broad sense[49]), such as hindbrain rhombomeres of vertebrates and reiterated pharyngeal slits of deuterostomes, can arise in evolution. Molecular and cellular studies of segmental patterning mechanisms across the animal kingdom and the nature of cycling gene networks are needed to tackle this issue. At present, therefore, we suggest that the common ancestor of ambulacrarians and chordates probably mixed the enteropneust character of pharyngeal slits and the annelid and chordate character of a centralized nerve cord. We cannot say with certainty that this ancestor was segmented along the body axis. Finally, some salient chordate characters seem to be novelties without precedents in either annelids or enteropneusts. For example, the organization of vertebrate muscle blocks working together with a notochord for active undulatory swimming[7]; this arrangement differs distinctively from the disposition of circular and longitudinal muscles in most other bilaterian animals.

If we now consider the next deepest node-based ancestor of the vertebrates, the urbilaterian ancestor to all bilateral animals, we can use the same logic as earlier, and many of the same data, to approach the reconstruction of the body plan. To pick up on just the three key morphological features discussed earlier — pharyngeal slits, a central nerve cord and segments — we deduce that it lacked pharyngeal slits and might have possessed a central nerve cord. However, there is too much uncertainty to decide whether it had segments along the body axis. It is reasonable to assume that the urbilaterian was unlike any animal alive today, but shared characters both with modern annelids and with modern enteropneusts. To turn this around, each of these two living groups seem to retain some of the characters from their, and our, distant ancestor.

We conclude, therefore, that the annelid and enteropneust scenarios are both partly correct. Some of the early proponents of the vast range of scenarios for the origin of vertebrates (Fig. 1) viewed living animals as proxies for long extinct ancestors. In reality, more progress has been made by comparing living animals with one another to deduce the combinations of morphological characters present in ancestors, a task that requires critical evaluation of homology, incorporating developmental, cellular and molecular approaches in an ever-widening range of animal taxa. Ultimately, a wealth of reliable and detailed information over a wide spectrum of taxa will be needed to sort out relationships among the animal phyla and their component characters[53,54]. This Review began with an appreciation of the older ideas in the field, and some of these will continue to guide us as we move ahead with technological advances and new discoveries in biology and palaeontology to gain insights into the origin of the vertebrates and our own distant history. ■

Received 22 September; accepted 24 November 2014.

1. Lamarck, J. B. *Philosophie Zoologique* [in French] Vol. 2 (Dentu, 1809).
The first explicitly evolutionary derivation of vertebrates from invertebrates.
2. Geoffroy St-Hilaire, E. Considérations générales sur la vertèbre [in French]. *Mém. Mus. Hist. Nat.* **9,** 89–119 (1822).
3. Patten, W. *The Evolution of the Vertebrates and Their Kin* (Blakiston's Son, 1912).
4. Gislén, T. Affinities between the Echinodermata, Enteropneusta, and Chordania. *Zool. Bidr. Uppsala* **12,** 199–304 (1930).
5. Jefferies, R. P. S. *The Ancestry of the Vertebrates* (British Museum Natural History, 1986).
This scholarly work, even if no longer accepted in its broad outline, was centrally important for reawakening interest in the invertebrate-to-vertebrate transition in the latter part of the twentieth century.
6. Satoh, N. An aboral-dorsalization hypothesis for chordate origin. *Genesis* **46,** 614–622 (2008).
7. Gans, C. & Northcut, R. G. Neural crest and the origin of the vertebrates: a new head. *Science* **220,** 268–274 (1983).
This influential scenario did much to focus the attention of vertebrate biologists back on questions about the evolutionary origin of their group.
8. Northcutt, R. G. The new head hypothesis revisited. *J. Exp. Zool.* **304B,** 274–297 (2005).
9. Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439,** 965–968 (2006).
10. Putnam, N. H. *et al*. The amphioxus genome and the evolution of the chordate karyotype. *Nature* **453,** 1064–1071 (2008).
11. Dohrn, A. *Der Ursprung der Wirbelthiere und das Princip des Functionswechsels: genealogische Skizzen* [in German] (Engelmann, 1875).
This is the original annelid theory.
12. Semper, C. Die Stammesverwandtschaft der Wirbelthiere und Wirbellosen [in German]. *Arb. Zool.-Zootom. Inst. Würzburg* **2,** 25–76 (1875).
13. Balavoine, G. & Adoutte, A. The segmented urbilateria: a testable scenario. *Integr. Comp. Biol.* **43,** 137–147 (2003).
14. Eisig, H. Der Nebendarm der Capitelliden und seine Homologa [in German]. *Zool. Anz.* **1,** 148–152 (1878).
15. Kleinenberg, N. Die Entstehung des Annelids aus der Larve von Lopadorhynchus, nebst Bemerkungen über die Entwicklung anderer Polychaeten [in German]. *Z. Wiss. Zool.* **44,** 1–227 (1886).
16. Van Beneden, E. & Julin, C. Recherches sur la morphologie des tuniciers [in French]. *Arch. Biol.* (Liege) **6,** 237–476 (1886).
17. Koehler, R. Sur la parenté du Balanoglossus [in French]. *Zool. Anz.* **9,** 506–507 (1886).
18. Beard, J. Some annelidan affinities in the ontogeny of the vertebrate nervous system. *Nature* **39,** 259–261 (1889).
19. Kennel, J. Ueber die Ableitung der Vertebratenaugen von den Augen der Anneliden [in German]. *Sitzungsber. Naturforsch. Ges. Univ. Dorpat.* **9,** 408–411 (1892).
20. Minot, C. S. Cephalic homologies. A contribution to the determination of the ancestry of the vertebrates. *Am. Nat.* **31,** 927–943 (1897).
21. Bernard, H. M. A new reading for the annulate ancestry of the Vertebrata. *Nat. Sci.* **13,** 17–30 (1898).
22. Jones, C. M., Lyons, K. M., Lapan, P. M., Wright, C. V. E. & Hogan, B. L. M. DVR-4 (bone morphogenetic protein-4) as a posteriorventralizing factor in *Xenopus* mesoderm induction. *Development* **115,** 639–647 (1992).
23. Arendt, D. & Nübler-Jung, K. Inversion of dorsoventral axis? *Nature* **371,** 26 (1994).
This publication launched the current revival of the annelid theory.
24. Holley, S. A. *et al*. A conserved system for dorso-ventral patterning in insects and vertebrates involving *sog* and *chordin*. *Nature* **376,** 249–253 (1995).
This application of developmental genetic data to classic questions of animal phylogeny attracted much attention to the young field of evolutionary developmental biology.
25. Arendt, D. & Nübler-Jung, K. Comparison of early nerve cord development in insects and vertebrates. *Development* **126,** 2309–2325 (1999).
26. Kulakova, M. *et al*. Hox gene expression in larval development of the polychaetes *Nereis virens* and *Platynereis dumerilii* (Annelida, Lophotrochozoa). *Dev. Genes Evol.* **217,** 39–54 (2007).
27. Denes, A. S. *et al*. Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in Bilateria. *Cell* **129,** 277–288 (2007).
28. Tomer, R., Denes, A. S., Tessmar-Raible, K. & Arendt, D. Profiling by image registration reveals common origin of annelid mushroom bodies and vertebrate pallium. *Cell* **142,** 800–809 (2010).
29. Lauri, A. *et al*. Development of the annelid axochord: insights into notochord evolution. *Science* **345,** 1365–1368 (2014).
30. Halanych, K. M. The new view of animal phylogeny. *Annu. Rev. Ecol. Evol. Syst.* **35,** 229–256 (2004).
31. Philippe, H. *et al*. Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. *Nature* **470,** 255–260 (2011).
32. Bateson, W. The ancestry of the Chordata. *Q. J. Microsc. Sci.* **26,** 535–571 (1886).
This article is the original enteropneust theory.
33. Masterman, A. T. On the Diplochorda. I. The structure of Actinotrocha. *Q. J. Microsc. Sci.* **40,** 281–338 (1897).
34. Nübler-Jung, K. & Arendt, D. Enteropneusts and chordate evolution. *Curr. Biol.* **6,** 352–353 (1996).
35. Lowe, C. J. *et al*. Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* **113,** 853–865 (2003).
This publication launched the current revival of the enteropneust theory.
36. Puelles, L. & Ferran, J. L. Concept of neural genoarchitecture and its genomic fundament. *Front. Neuroanat.* **6,** 47 (2012).
37. Lowe, C. J. Molecular genetic insights into deuterostome evolution from the direct-developing hemichordate *Saccoglossus kowalevskii*. *Phil. Trans. R. Soc. B* **363,** 1569–1578 (2008).
38. Nomaksteinsky, M. *et al*. Centralization of the deuterostome nervous system predates chordates. *Curr. Biol.* **19,** 1264–1269 (2009).
39. Cunningham, D. & Casey, E. S. Spatiotemporal development of the embryonic nervous system of *Saccoglossus kowalevskii*. *Dev. Biol.* **386,** 252–263 (2014).
40. Duboc, V., Röttinger, E., Lapraz, F., Besnardeau, L. & Lepage, T. Left-right asymmetry in the sea urchin embryo is regulated by nodal signaling on the right side. *Dev. Cell* **9,** 147–158 (2005).
41. Wlizla, M. Evolution of Nodal signaling in Deuterostomes: insights from *Saccoglossus kowalevskii*. PhD thesis, Univ. Chicago (2011).
42. Röttinger, E., Duboc, T. & Martindale, M. Q. Investigating the role of the Nodal signaling pathway in a indirect developing hemichordate, *Ptychodera flava*. *Integr. Comp. Biol.* **50,** abstract, e144 (2010).

43. Miyamoto, N. & Wada, H. Hemichordate neurulation and the origin of the neural tube. *Nature Commun.* **4,** 2713 (2013).
44. Gaskell, W. H. *et al*. Origin of vertebrates. *Proc. Linn. Soc. Lond.* **122,** 9–50 (1910).
45. Ota, K. G., Fujimoto, S., Oisi, Y. & Kuratani, S. Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. *Nature Commun.* **2,** 373 (2011).
46. Furlong, R. F. & Holland, P. W. H. Bayesian phylogenetic analysis supports monophyly of ambulacraria and of cyclostomes. *Zoolog. Sci.* **19,** 593–599 (2002).
47. Achatz, J. G., Chiodin, M., Salvenmoser, W., Tyler, S. & Martinez, P. The Acoela: on their kind and kinships, especially with nemertodermatids and xenoturbellids (Bilateria incertae sedis). *Org. Divers. Evol.* **13,** 267–286 (2013).
48. Ogasawara, M., Wada, H., Peters, H. & Satoh, N. Developmental expression of *Pax1/9* genes in urochordate and hemichordate gills: insight into function and evolution of the pharyngeal epithelium. *Development* **126,** 2539–2550 (1999).
49. Graham, A., Butts, T., Lumsden, A. & Kiecker, C. What can vertebrates tell us about segmentation? *EvoDevo* **5,** 24 (2014).
50. De Robertis, E. M. The molecular ancestry of segmentation mechanisms. *Proc. Natl Acad. Sci. USA* **105,** 16411–16412 (2008).
51. Dray, N. *et al*. Hedgehog signaling regulates segment formation in the annelid Platynereis. *Science* **329,** 339–342 (2010).
52. Beaster-Jones, L. *et al*. Expression of somite segmentation genes in amphioxus: a clock without a wavefront? *Dev. Genes Evol.* **218,** 599–611 (2008).
53. Wagner, G. P. The developmental genetics of homology. *Nature Rev. Genet.* **8,** 473–479 (2007).
54. Northcutt, R. G. Evolution of centralized nervous systems: two schools of evolutionary thought. *Proc. Natl Acad. Sci. USA* **109** (Suppl. 1), 10626–10633 (2012).
55. van Wijhe, J. W. Über den vorderen neuroporus und die phylogenetische function des canalis neurentericus der wirbelthiere [in German]. *Zool. Anz.* **7,** 683–687 (1884).
56. Ziegler, H. E. Die phylogenetische entstehung des kopfes der wirbeltiere [in German]. *Jena. Zeitschr. Naturwiss.* **43,** 653–684 (1908).
57. Bjerring, H. C. Major anatomical steps toward craniotedness: a heterodox view based largely on embryological data. *J. Vert. Paleontol.* **4,** 17–29 (1984).
58. Hatschek, B. Studien über Entwicklungsgeschichte der Anneliden. Ein beitrag zur morphologie der Bilaterien [in German]. *Arb. Zool. Inst. Wien* **11,** 1–128 (1878).
59. Roule, L. Étude sur les forms premièrs de la notochorde et sur les affinitiés naturelles des cordés [in French]. *Arch. Zool. Exp. Gén.* (Sér.4) **10,** 447–547 (1909)
60. Delsman, H. C. *The Ancestry of Vertebrates* (Valkoff, 1922).
Feelings ran high about vertebrate-origin scenarios, for instance, J. W. van Wijhe thought this work "ought to be confiscated and consigned to the flames".
61. Marlow, H. *et al*. Larval body patterning and apical organs are conserved in animal evolution. *BMC Biol.* **12,** 7 (2014).
62. Garstang, W. Preliminary note on a new theory of the phylogeny of the Chordata. *Zool. Anz.* **17,** 122–125 (1894).
63. Jollie, M. The origin of chordates. *Acta Zool. Stockh.* **54,** 81–100 (1973).
64. Ivanova-Kazas, O. M. On the ancestry of Chordata and Deuterostomia as a whole. *Russ. J. Mar. Biol.* **23,** 219–226 (1997).
65. Nielsen, C. *Animal Evolution: Interrelationships of the Living Phyla* (Oxford Univ. Press, 2001).
66. Kupffer, C. v. Die stammverwandtschaft zwischen Ascidien und wirbelthieren [in German]. *Ark. Mik. Anat.* **6,** 115–172 (1870).
67. Garstang, W. The morphology of the Tunicata, and its bearings on the phylogeny of the Chordata. *Q. J. Microsc. Sci.* **72,** 51–187 (1928).
68. Berrill, N. J. *The Origin of Vertebrates* (Clarendon, 1955).
69. Whitear, M. Some remarks on the ascidian affinities of vertebrates. *Ann. Mag. Nat. Hist.* (Ser. 12) **10,** 338–348 (1957).
70. Romer, A. S. *The Vertebrate Body* 3rd Edn (Saunders, 1962).
The scenario of Romer became well known because his book was read attentively by generations of American premedical students cramming for medical school acceptance.
71. Berg, L. S. *Nomogenesis or Evolution Determined by Law* (Constable, 1926).
72. Clark, A. H. *The New Evolution: Zoogenesis* (Williams & Wilkins, 1930).
73. Nursall, J. R. On the origin of the major groups of animals. *Evolution* **16,** 118–123 (1962).
74. Anderson, D. T. Origins and relationships among animal phyla. *Proc. Linn. Soc. N. S. W.* **106,** 151–166 (1982).
75. Sedgwick, A. On the origin of metameric segmentation and some other morphological questions. *Q. J. Microsc. Sci.* **24,** 43–82 (1884).
76. Lameere, A. L'origine des vertébrés [in French]. *Bull. Séanc. Soc. Belge Micros.* **17,** 91–121 (1891).
77. Inglis, W. G. Evolutionary waves: patterns in the origins of animal phyla. *Aust. J. Zool.* **33,** 153–178 (1985).
78. Dewel, R. A. Colonial origin for Eumetazoa: major morphological transitions and the origin of bilaterian complexity. *J. Morphol.* **243,** 35–74 (2000).
79. Tretjakoff, D. Ursprung der Chordaten [in German]. *Z. Wiss. Zool.* **134,** 558–640 (1929).
80. Christofersen, M. L. & Araujo-de-Almeida, E. A phylogenetic framework of the Enterocoela (Metameria: Coelomata). *Rev. Nordest. Biol.* **9,** 173–208 (1994).
81. Gerhart, J. & Kirschner, M. *Cells, Embryos and Evolution: Toward a Cellular and Developmental Understanding of Phenotypic Variation and Evolutionary Adaptability* (Blackwell, 1997).
82. Leydig, F. *Vom Bau des thierischen Körpers. Handbuch der vergleichenden Anatomie*, Vol. 1 [in German] (Laupp & Siebeck, Tübingen, 1864).
This is the first scenario for an invertebrate-to-vertebrate transition to appear after *On the Origin of Species* and to be written from a Darwinian point of view.
83. Gaudry, A. *Les Enchaînments du Monde Animal dans les Temps Géologiques* [in French] (Savy, 1883).
84. Jaeckel, O. Über die Stammform der Wirbelthiere [in German]. *Sitzungsber. Ges. Naturforsch. Freunde Berlin* **1896,** 107–129 (1896).
85. Gaskell, W. H. *The Origin of Vertebrates* (Longmans Green, 1908).
86. Raw, F. Outline of a theory of origin of the Vertebrata. *J. Paleontol.* **34,** 497–539 (1960).
87. Sillman, L. R. The origin of the vertebrates. *J. Paleontol.* **34,** 540–544 (1960).
88. Hoffhaus, C. E. A homogeneous theory of the origin of vertebrates. *J. Paleontol.* **37,** 458–471 (1963).
89. Løvtrup, S. *The Phylogeny of Vertebrata* (Wiley, 1977).
90. Bergström, J. The origin of animal phyla and the new phylum Procoelomata. *Lethaia* **22,** 259–269 (1989).
91. Hesse, R. *Tierbau und Tierleben in Ihrem Zusammenhang Betrachtet. Vol. I. Der Tierkörper als Selbständiger Organismus* [in German] (Teubner, 1910).
92. Naef, A. Notizen zur morphologie und stammgeschichte der wirbeltiere. 7. Das verhältnis der chordaten zu niederen tierformen und der typische verlauf ihrer frühen entwicklung [in German]. *Biol. Zentralbl.* **46,** 39–50 (1926).
93. Sepp, E. K. *Developmental History of the Nervous System of Vertebrates* [in Russian] (Medgiz, 1959).
94. Engelbrecht, D. V. Z. The annelid ancestry of the chordates and the origin of the chordate central nervous system and the notochord. *J. Zool. Syst. Evol. Res.* **7,** 18–30 (1969).
95. Gutmann, W. F. Relationships between invertebrate phyla based on functional-mechanical analysis of the hydrostatic skeleton. *Am. Zool.* **21,** 63–81 (1981).
96. Hubrecht, A. A. W. The relation of the Nemertea to the Vertebrata. *Q. J. Microsc. Sci.* **27,** 605–644 (1887).
97. Macfarlane, J. M. *The Causes and Course of Organic Evolution. A Study in Bioenergetics* (Macmillan, 1918).
98. Jensen, D. D. Hoplonemertines, myxinoids and deuterostome origins. *Nature* **188,** 649–650 (1960).
99. Willmer, E. N. Nemertines as possible ancestors of the vertebrates. *Biol. Rev. Camb. Philos. Soc.* **49,** 321–363 (1974).
100. Dzik, J. The origin of the mineral skeleton in chordates. *Evol. Biol.* **31,** 105–154 (2000).
101. Goette, A. Über den Ursprung der Wirbelthiere [in German]. *Verh. Dtsch. Zool. Ges.* **5,** 12–30 (1895).
102. Plate, L. Über den Ursprung der Wirbelthiere; eine kritische Besprechung [in German]. *Anat. Anz.* **58,** 39–46 (1924).
103. Salvini-Plawen, L. The urochordate larva and archicoelomate organization: chordate origin and anagenesis revisited. *J. Zool. Syst. Evol. Res.* **36,** 129–145 (1998).
104. Gregory, W. K. The transformation of organic design: a review of the origin and deployment of the earlier vertebrates. *Biol. Rev.* **11,** 311–344 (1936).
105. Dillon, L. S. The hydrocoel and the ancestry of the chordates. *Evolution* **19,** 436–446 (1965).
106. Eaton, T. H. The stem-tail problem and the ancestry of the chordates. *J. Paleontol.* **44,** 969–979 (1970).
107. Kuznetsov, A. N. Five longitudes in chordate body. *Theor. Biol. Forum* **105,** 21–35 (2012).
108. Béraneck, M. E. *Théories Récentes Sur la Descendance des Vertébrés* [in French] (Attinger, 1892).
109. Theophiloff, S. *Zur Phylogenie der Tunicaten: Eine Kritische Studie* [in German]. PhD thesis, Univ. Jena (1892).
110. MacBride, E. W. A review of Professor Spengel's monograph on Balanoglossus. *Q. J. Microsc. Sci.* **36,** 385–420 (1894).
111. Kemna, A. L'origine de la corde dorsale [in French]. *Ann. Soc. Roy. Zool. Malacol. Belg.* **39,** lxxxv–clvii (1904).
112. van der Horst, C. J. Hemichordata in *Dr H. G. Bronn's Klassen und Ordnungen des Tierreichs* [in German]. Vol. 4, Part 4, Book 2, Section 2, Installments 1–5 (Akademische Verlagsgesellschaft, 1939).
113. Tokioka, T. Phylogenetic speculation of the Tunicata. *Publ. Seto Mar. Biol. Lab.* **19,** 43–63 (1971).
114. Cameron, C. B., Garey, J. R. & Swalla, B. J. Evolution of the chordate body plan: new insights from phylogenetic analysis of deuterostome phyla. *Proc. Natl Acad. Sci. USA* **97,** 4469–4474 (2000).
115. Röttinger, E. & Lowe, C. J. Evolutionary crossroads in developmental biology: hemichordates. *Development* **139,** 2463–2475 (2012).
116. Ayers, H. Concerning vertebrate cephalogenesis. *J. Morphol.* **4,** 221–245 (1890).
117. Willey, A. *Amphioxus and the Ancestry of the Vertebrates* (Macmillan, 1894).
118. Perrier, E. L'origine des vertébrés. *C. R. Acad. Sci. Paris* **126,** 1479–1486 (1898).
119. Manzanares, M. & Nieto, M. A. A celebration of the new head and an evaluation of the new mouth. *Neuron* **37,** 895–898 (2003).
120. Todaro, F. Sur l'origine phylogénétique des yeux des vertébrés et sur la signification des épiphises et des hypophyses de leur cerveau [in French]. *Arch. Ital. Biol.* **9,** 55–57 (1888).
121. Brooks, W. K. The genus Salpa. *Mem. Biol. Lab. Johns Hopkins Univ.* **2,** 1–303 (1893).
122. Delage, Y. & *Hérouard, E. Traité de Zoologie Concrète. Vol VII. Les Procordés* [in French] (Schleicher, 1898).
123. Sewertzoff, A. N. Directions of evolution. *Acta Zool.* **10,** 59–141 (1929).
124. Wada, H. Evolutionary history of free-swimming and sessile lifestyles in urochordates as deduced from 18S rDNA molecular phylogeny. *Mol. Biol. Evol.* **15,** 1189–1194 (1998).
125. Halanych, K. M. *et al*. Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science* **267,** 1641–1643 (1995).
This pioneering study in molecular phylogenetics indicated that the greater part of the animal kingdom is divisible into three major super-phyletic groups.

Acknowledgements We are grateful to R. Jenner and T. Lacalli for their criticisms during preparation of our manuscript. We dedicate this Review to the memory of Yoshiki Sasai, whose elucidation of the genetic control of dorsoventral patterning will endure as a landmark in the field of evolutionary developmental biology.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing interests. Readers are welcome to comment on the online version of this paper at go.nature.com/y7dnfd. Correspondence should be addressed to N.D.H. (nholland@ucsd.edu).

REVIEW

doi:10.1038/nature14434

The deuterostome context of chordate origins

Christopher J. Lowe[1], D. Nathaniel Clarke[1], Daniel M. Medeiros[2], Daniel S. Rokhsar[3,4,5] & John Gerhart[3]

Our understanding of vertebrate origins is powerfully informed by comparative morphology, embryology and genomics of chordates, hemichordates and echinoderms, which together make up the deuterostome clade. Striking body-plan differences among these phyla have historically hindered the identification of ancestral morphological features, but recent progress in molecular genetics and embryology has revealed deep similarities in body-axis formation and organization across deuterostomes, at stages before morphological differences develop. These developmental genetic features, along with robust support of pharyngeal gill slits as a shared deuterostome character, provide the foundation for the emergence of chordates.

The mystery of chordate origins has endured for more than 150 years. Shortly after Darwin's *On the Origin of Species*, acorn worms were discovered to have chordate-like pharyngeal gill slits[1,2] and to metamorphose from echinoderm-like larva[3], thus linking the evolution of chordates, hemichordates and echinoderms. Modern phylogenetic analysis has confirmed the union of these three phyla in a single clade. This group, the deuterostomes, provides the phylogenetic framework for developing hypotheses about the origin of chordate features through comparative morphology, embryology and genomics.

The emergence of comparative molecular developmental biology over the past quarter of a century has revived interest in classic hypotheses of animal body-plan evolution[4]. The comparative approach focuses on identifying morphological, developmental and genetic traits that are shared across phyla by virtue of their inheritance from a common ancestor, and provides an understanding of how such ancestral traits can arise and be subsequently modified. Although many recent hypotheses on chordate and vertebrate origins on the basis of molecular data are motivated primarily by projections from the bilaterian ancestor[4,5], a growing body of data from hemichordates, echinoderms and invertebrate chordates serves as the foundation for new hypotheses based on deuterostome ancestral characters[6–14].

Despite the impressive morphological disparity among deuterostome phyla, we are making progress identifying conserved anatomical and molecular ancestral characters. Each phylum is a fascinating natural experiment in body-plan evolution, but their dazzling diversity presents a major challenge for reconstructing early deuterostome evolutionary history in morphological terms (Box 1)[15]. In this Review we highlight recent advances in deuterostome phylogenetics, developmental biology and genomics that have contributed to our understanding of the early evolution of deuterostomes and the subsequent origin of chordates.

Deuterostome phylogeny

The first step in unravelling chordate origins is the establishment of a robust deuterostome phylogeny (Fig. 1). The chordates, uniting vertebrates, tunicates and cephalochordates, were first recognized by Haeckel[16], partly based on shared developmental characteristics. A key insight came from Kowalevsky's[17] recognition that the tadpole larva of ascidians shared many characteristics with vertebrates, an observation that greatly impressed Darwin[18]. Kowalevsky also recognized the vertebrate-like gill slits of the invertebrate acorn worms[2]. The link between chordates and acorn worms was emphasized by Bateson, who proposed further morphological affinities between them in the late 1800s, and named the acorn worms 'hemichordates'[1]. Around the same time, Metchnikoff recognized the similar larval forms of hemichordates and echinoderms, and united these two phyla into the 'Ambulacraria'[3] (Box 2).

The unity of chordates, hemichordates and echinoderms was inferred by Grobben[19] on the basis of three shared developmental features: 'deuterostomous' development (derivation of the mouth from a secondary opening rather than the blastopore), radial cleavage and enterocoely (the pouching out of mesoderm from the archenteron wall). Although he named this lineage the 'deuterostomes' (second mouth), we now recognize that these features are not unique to the chordate–hemichordate–echinoderm clade, and are found in several other phyla[20], the result of either shared ancestry or convergence. This leads to the nomenclatural embarrassment that some phyla with deuterostomous development are not deuterostomes. Nevertheless the name has stuck, and by convention we refer to the chordate–hemichordate–echinoderm clade as the deuterostomes.

The advent of molecular phylogenomics has brought new methods to bear on the relationships between and within deuterostome phyla (Fig. 1). Ambulacraria, the surprising grouping of hemichordates and echinoderms, is strongly supported by molecular characters[15,21–23], and is clearly the sister group of chordates. Within chordates, it is now widely recognized that the cephalochordate lineage (amphioxus) diverged before the split between tunicates and vertebrates[21,24]. This recent discovery overturned earlier thinking that tunicates diverged first, which had implied that the simple ascidian tadpole larva represents ancestral chordate features (Box 1).

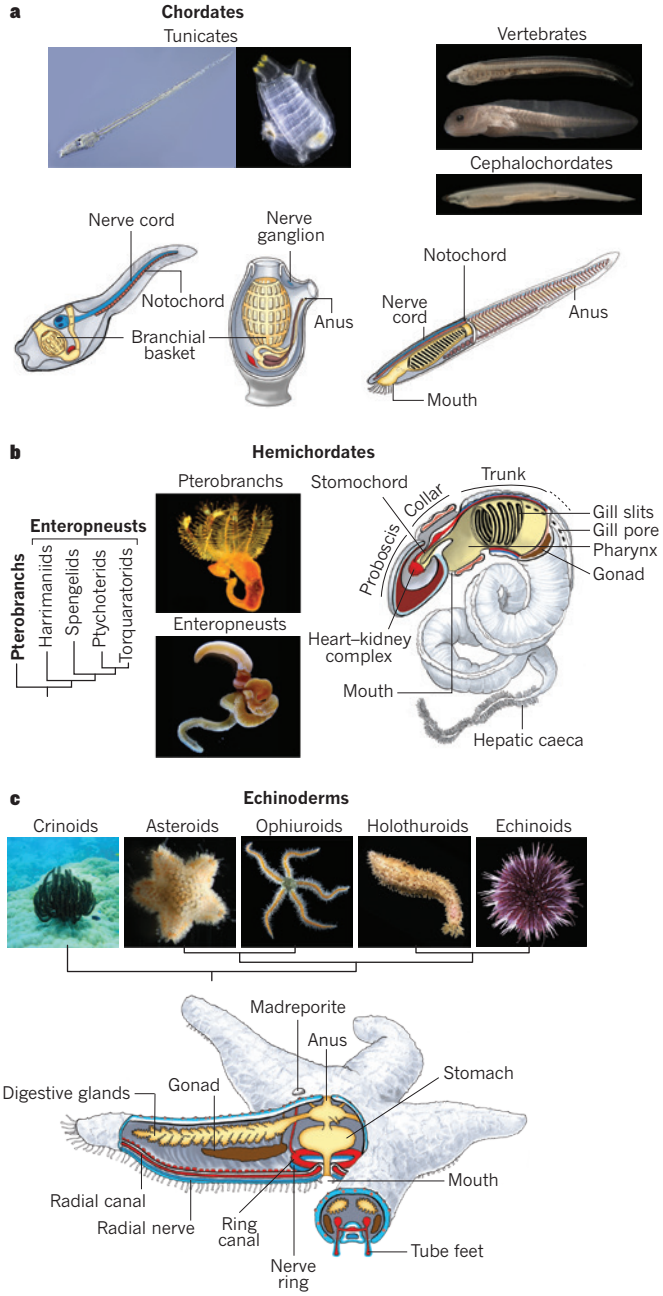
Although classic embryological criteria suggested that lophophorates (phoronids, brachiopods and bryozoans) and/or chaetognaths should also be grouped among the deuterostomes, molecular phylogenetics robustly supports their position in the protostomes[22,25,26]. Xenoturbellid worms are a more challenging case: these animals resemble acoelomorphs (acoel flatworms and nematodermatids) and have been grouped with them in a 'Xenacoelomorpha' clade[27,28]. Some molecular analyses also identify *Xenoturbella* and its relatives as ambulacrarians, and therefore deuterostomes[27], whereas other studies find that acoelomorphs diverge from

[1]Hopkins Marine Station, Department of Biology, Stanford University, 120 Oceanview Boulevard, Pacific Grove, California 93950, USA. [2]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder Ramaley N122 Campus Box 334 Boulder, Colorado 80309-0334, USA. [3]Department of Molecular and Cell Biology University of California, Berkeley 142 Life Sciences Addition 3200 Berkeley, California 94720-3200, USA. [4]Okinawa Institute of Science and Technology 1919-1 Tancha, Onna, Okinawa Prefecture 904-0495, Japan. [5]Department of Energy Joint Genome Institute 2800 Mitchell Drive Walnut Creek, California 94598, USA.

BOX 1

Deuterostome diversity

Unambiguous homologies between deuterostome phyla with morphologically disparate body plans are difficult to establish, leading to a wide range of often contradictory hypotheses about chordate origins[1,6,29,96,100,107,108,114–116]. We present basic descriptions of the adult body plans of the uncontested deuterostome phyla: chordates, hemichordates and echinoderms. All mesodermally derived-structures are red, ectoderm are blue and endoderm are yellow. **a**, Chordates are set apart from other deuterostomes by a suite of features that enable swimming by paired muscles along a trunk that extends post-anally. These muscles exert forces on the notochord, a flexible rod that provides elastic recoil to power movement. Chordates also have a unique tubular central nervous system (CNS)[29]. Of the subphyla, vertebrates are distinguished from other chordates by the elaboration of the head region with an enlarged anterior CNS with paired sense organs, evident here in a lamprey ammocoete larva (top) and an axolotl tadpole (bottom). Tunicates (larvaceans, ascidians and thalacians) are a diverse group of marine filter feeders that display a range of body plans and life-history strategies, including solitary, colonial, sessile and free-swimming forms[117]. They are represented here by ascidians. Chordate affinities are most evident in the larval form: an ascidian tadpole (left) has a tubular nerve cord, a notochord and a post-anal tail. These features regress at metamorphosis, leaving the branchial basket, a small nerve ganglion and the endostyle as the only chordate characters remaining in the adult (right). Cephalochordates, represented by amphioxus, are filter feeders that burrow in sand with their mouths open to the water column. Amphioxus shares much of its basic anatomy with vertebrates, including segmented musculature, and a vertebrate-like heart and circulatory system[30,118]. They have a modest CNS consisting of a neural tube with simplified vertebrate-type patterning along both the anteroposterior and dorsoventral axes[8,118,119]. **b**, Hemichordates are a clade of marine worms divided into two groups: enteropneusts and pterobranchs. Hemichordate phylogeny is based on Cannon *et al.*[120]. Pterobranchs, shown here by *Cephalodiscus*, are small largely colonial animals that live within the protection of a secreted fibrous tube and use a ciliated lophophore for filter feeding[50,58]. Enteropneusts, or acorn worms, are solitary, burrowing worms that feed using a combination of deposit and filter feeding[52,121]. The harrimaniid *Saccoglossus kowalevskii*, which has been used for many developmental studies[12], is pictured (micrograph). Both groups of hemichordates are united by their tripartite body plan, which includes proboscis, collar and trunk (as shown in the illustration of a spengelid entropneust). The proboscis is used for digging and feeding and contains the gut diverticulum called the stomochord that supports a heart–kidney complex[56,60]. The mouth opens ventrally into the pharynx within the collar region, and the anterior trunk is perforated by a series of dorsolateral gill slits[58]. **c**, Echinoderms have considerably modified the ancestral bilaterian body plan to become pentaradially symmetrical as adults, although their larvae are bilaterally symmetric (Box 2). Even basic axis comparisons with other deuterostomes are problematic, and the evolutionary origins of this phylum remain a mystery. All five extant classes of echinoderms: crinoids (sea lilies), asteroids (sea stars), ophiuroids (brittle stars), holothuroids (sea cucumbers) and echinoids (sea urchins) are characterized by a conserved body plan shown by a diagram of an asteroid with cutaways to show internal anatomy; the mesodermally derived water vascular system, a hydraulic system that drives the distinctive tube feet used for feeding and locomotion; five radial nerves along each arm/ambulacrum linked by a nerve ring, and the mesodermally derived skeleton. Asteroids most clearly exhibit the basic components of the body plan. Phylogenetic relationships are based on refs 120, 122.



the bilaterian stem before the protostome–deuterostome split[28] (Fig. 1). We note, however, that even if xenoturbellids and/or acoelomorphs are deuterostomes, their simple body plans would represent secondary loss from a more complex deuterostome ancestor. The resolution of the phylogenetic placement of these taxa is therefore unlikely to provide substantial insight into vertebrate origins.

Ancestral chordate characters

On the basis of shared features of living chordates we have gained a rather detailed view of the development, morphology and life history of the last common chordate ancestor. Most classic and modern reconstructions of ancestral chordates propose a filter feeder with a notochord, gill slits, endostyle, dorsal hollow nerve cord and post-anal tail[29]. The recent

revision of the chordate family tree has added to this list of ancestral chordate features. The basal position of cephalochordates among chordates suggests that similarities between amphioxus and vertebrates represent ancestral chordate features lost in tunicates[21,24]. Thus, in addition to the core features listed earlier, the Early Cambrian or Pre-Cambrian chordate ancestor probably possessed myomeres, a vertebrate-like circulatory system and a central nervous system (CNS)[30]. The life history of cephalochordates, and the fact that larval lampreys and adult hemichordates are burrowing filter feeders, further suggest that this ancestor was a solitary, endobenthic filter feeder that was capable of short swims.

The striking similarities between amphioxus- and vertebrate-developmental mechanisms allow a fairly comprehensive reconstruction of early development in primitive chordates. As in vertebrates and cephalochordates, the anteroposterior (AP) and dorsoventral (DV) axes of the ancestral chordate were probably determined during gastrula stages by organizing centres much like Spemann's organizer of vertebrates, secreting long-range patterning signals[11]. Opposing Nodal and BMP signalling gradients established the DV axis, with Chordin-mediated BMP inhibition in the dorsal ectoderm segregating the presumptive CNS from the epidermal (or general) ectoderm[11,31]. Along the AP axis, Wnt and retinoic acid signalling probably acted on Hox genes and other transcription-factor genes to establish the regional identities of AP domains of the body axis, including the boundary between the foregut and hindgut and the main subdivisions of the CNS[8,32,33].

Comparisons between amphioxus and vertebrates suggest a deep ancestry of the major divisions of the CNS along the AP axis. Later in development, fine-scale patterning of the ancestral chordate CNS was also vertebrate-like, but simpler. Along the DV axis of the CNS, all chordates have a molecularly distinct dorsal domain that expresses *pax3/7*, *msx* and *zic* genes and generates sensory interneuron cells[34], a ventral floor plate expressing hedgehog ligands[35], and an intervening bilateral domain flanking the neural tube lumen and generating motor and visceral neurons. The expression domains of transcription factors and signalling molecules along the AP axis of the CNS are also mostly conserved across chordates, and presumably reflect expression domains of the chordate ancestor[8]. Precisely how this patterning was generated is less clear, as current data suggest that neither amphioxus nor tunicates have unambiguous, functionally validated homologues of two vertebrate CNS signalling centres, the isthmic organizer or the zona limitans intrathalamica (although these signalling mechanisms may have been present in a deuterostome ancestor, see later)[8,36,37].

Segmented musculature of the ancestral chordate almost certainly developed from somites, and at least some formed by enterocoely[35,38]. In amphioxus, the anterior-most somites form by enterocoely, whereas posterior somites pinch off sequentially from the tail bud[36,39]. In vertebrates, a 'clock and wavefront' mechanism, involving oscillating Notch and Wnt signalling and a posterior fibroblast growth factor (FGF)-signalling gradient divides the paraxial mesoderm into a series of somites[40]. Despite these mechanistic differences, amphioxus displays vertebrate-like segmental expression of Notch and Wnt signalling components in nascent somites, and requires FGF signalling for forming and maturing the anterior and posterior somites[41,42]. Thus, somitogenesis in all living chordates, and presumably their last common ancestor, involved iterated Notch–Delta and Wnt signalling, and FGFs.

Despite differences in when and how the pharyngeal gill slits form in the three chordate clades, recent work reveals conserved aspects of their development, presumably inherited from the chordate common ancestor. In amphioxus and vertebrates, the pharyngeal endoderm is specified by attenuated retinoic acid signalling, and marked by conserved expression of several transcription factors including pax1/9, six1/2, six4/5, six3/6, eya, foxC and foxL1 (refs 32, 43, 44). In addition, recent work has shown that the chordate ancestor probably had a collagen-based pharyngeal skeleton incorporating cellular[45] and acellular cartilage[46,47] derived from pharyngeal mesoderm. Whereas the pharyngeal walls develop pharyngeal pouches and gill slits, the floor develops endostyle specializations related to trapping food particles during filter feeding, as well as to hormonal and protective functions.

The deuterostome roots of chordate characteristics

Work on hemichordates and echinoderms has informed our understanding of ancestral deuterostome features, with different taxa contributing complementary insights. Integrating insights from echinoderms is challenging owing to the divergent radial body plan of adults, although studies of echinoderm larval development have made essential contributions to our understanding of early deuterostome embryogenesis[13,14,48,49]. Pterobranch hemichordates are relatively understudied[50]. In this Review, therefore, we focus primarily on insights derived from the study of enteropneust hemichordates (acorn worms) as they relate to our understanding of early deuterostome evolution.

As first described by Kowalevsky[2], the anterior gut of hemichordates is perforated in the dorsolateral region by a series of ciliated gill slits, now known to be supported by gill bars composed of an acellular collagen secreted by the endoderm (Fig. 2a, Box 1)[46]. Although there is no equivalent structure in extant echinoderms, fossils reveal compelling evidence that gill slits were present in stem echinoderms and subsequently lost[51]. On the basis of morphological and functional criteria, enteropneust gill slits closely resemble those of cephalochordates and are plausibly homologous[1,46,52]. In two species of enteropneust, studies of patterning genes with conserved roles in chordate gill-pouch development, namely *pax1/9*, *foxC*, *foxL1*, *eya*, *six1* and *foxI*, also strongly support homology[53–55].

The stomochord in hemichordates has drawn much comparative interest as a notochord-like ancestral trait[1,29,56,57]. It is a diverticulum of

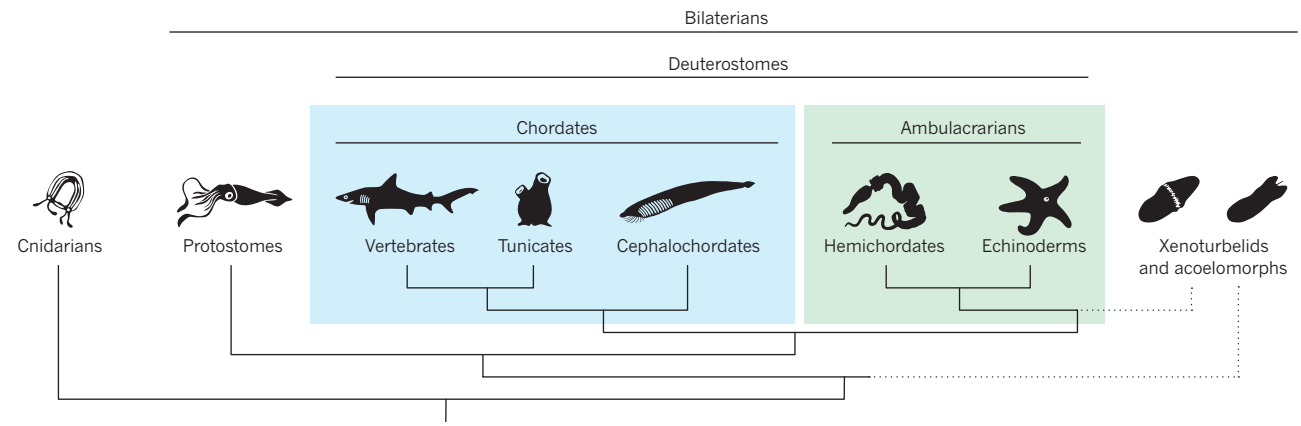


Figure 1 | Deuterostome phylogeny. A consensus cladogram of deuterostome groups based on recent phylogenomic data sets[21,22,24,28,113]. There are three major phyla of extant deuterostomes, which are grouped into two diverse clades: the ambulacrarian phyla (green), consisting of hemichordates and echinoderms, and chordates (blue), consisting of the cephalochordate, tunicate and vertebrate lineages. Recent analyses have proposed either a grouping of xenoturbellid and acoelomorph flatworms as sister group to ambulacrarians[27], or at the base of the bilaterians[28](dashed lines).

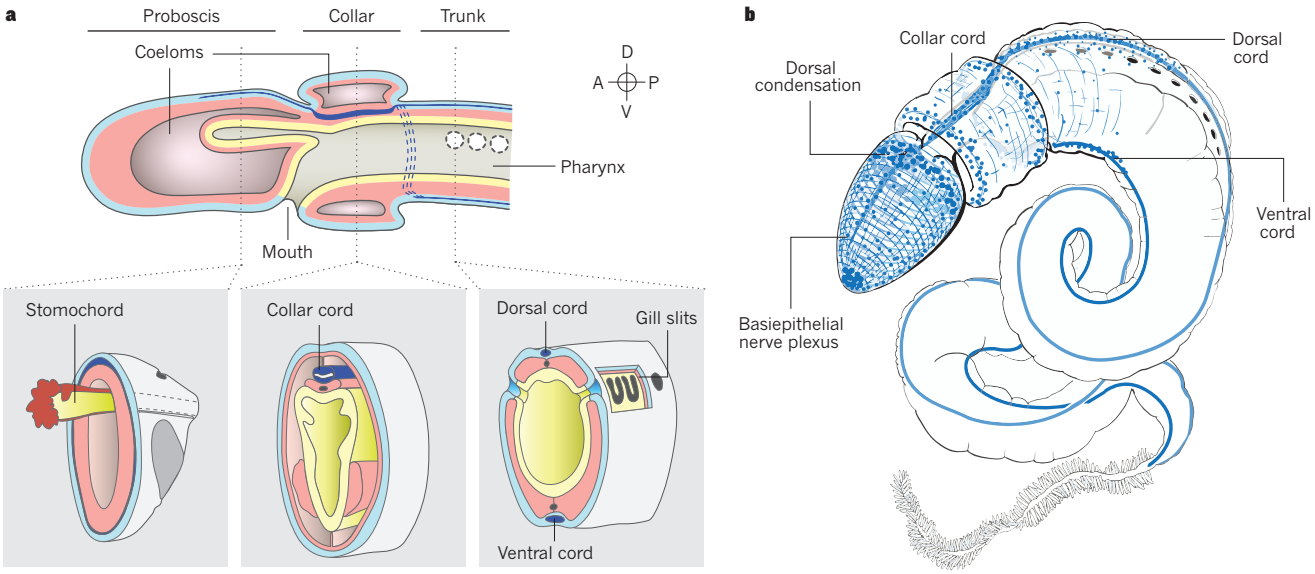


Figure 2 | Key anatomical features of the enteropneust body plan. **a**, Longitudinal and transverse sections through an adult enteropneust hemichordate, highlighting morphological characters that have featured prominently in classic hypotheses of deuterostome evolution and chordate origins. A, anterior; P, posterior; D, dorsal; V, ventral. **b**, The nervous system of an adult enteropneust showing both the broad basiepithelial plexus throughout the ectoderm and nerve chords along the dorsal and ventral midlines. The blue spots represent cell bodies and the lines represent neural processes.

the anterior gut that extends into the posterior proboscis supporting the heart–kidney complex on its dorsal surface (Fig. 2a, Box 1). Stomochord cells are vacuolated and surrounded by a sheath, similar in tissue organization to a notochord[1,56,58]. However, homology of these two structures is weakly supported by both morphological and molecular criteria[59–61]. In chordates the developing notochord is a key source of the secreted BMP antagonists Chordin, Noggin and Follistatin, and the ventralizing ligand Shh[62]. Of these genetic markers, only *hh* (the homologue of *Shh*) is expressed in the stomochord, but it is also observed in surrounding anterior endoderm[57,63]. Possible alternatives to notochord homology are suggested by the stomochord expression of genes such as *otx*, *dmbx*, *hex* and *foxE* that are expressed in prechordal endomesoderm of chordates, but not in the notochord. These markers suggest that the stomochord is an anterior endodermal structure with stronger affinities to the endostyle than the notochord[61].

The hemichordate nervous system is characterized by two contrasting organizational features (Fig. 2b): a broad basiepithelial plexus, particularly prominent in proboscis ectoderm, and a pair of nerve cords. The ventral cord extends the length of the trunk and the dorsal cord runs from the base of the proboscis down the length of the animal and joins to the ventral cord by lateral nerve rings. Both cords are superficial condensations of the nerve plexus except in a short length that spans the collar, where the cord is internalized into a tube with a prominent lumen in some species, and is formed by a developmental process that resembles chordate neurulation[59,64–67]. Various authors have proposed both cords as possible homologues of the chordate dorsal cord[57,59,68,69], however, the internalized collar cord has attracted the most attention[6,57,59,69]. Early reports suggested that the dorsal cord was simply a through conduction tract of axons[70,71]. Molecular studies, however, have shown condensations of cell bodies associated with this cord[6,69], and a further study in *Balanoglossus simodensis* revealed *bmp2/4*, *pax3/7* and *msx* expression in the collar cord[57], similar to that of the most lateral parts of the vertebrate neural plate and in other bilaterians during CNS development. Although these similarities are supportive of homology of the collar cord and chordate nerve cord, other neural molecular markers complicate this interpretation. In *Saccoglossus kowalevskii*, markers of medial rather than lateral neural plate are not expressed in the dorsal cord as predicted, but rather along the ventral midline associated with the ventral cord. In addition, several neural markers are not only expressed in the collar cord, but also throughout the length of the superficial cord in the trunk, suggesting a patterning role throughout the dorsal midline[72]. When considering the general organization of the nervous system in enteropneusts, no simple homology statements can yet be made in relation to other nervous systems.

Although it seems likely that ancestral deuterostomes inherited some elements of nervous system centralization from the bilaterian common ancestor, a comprehensive characterization of key molecular markers is needed to test competing hypotheses of nervous system evolution further. It remains unclear whether the main features of the unusual enteropneust nervous system can be ascribed to the filter-feeding deuterostome ancestor, thereafter modified in the chordate line, or whether they are secondary derivatives of the hemichordate lineage.

Axial patterning of deuterostome body plans

The discovery of conserved, pan-bilaterian mechanisms for the development of the animal–vegetal, AP and DV body axes has transformed our thinking about animal evolution[4,5,73]. This deep conservation initially surprised biologists because of the great morphological diversity of bilaterians, but made more sense when it was realized that the early axiation processes of the embryo are separate from the later processes of morphogenesis, organogenesis and cell differentiation. Conserved suites of genes are responsible for establishing basic regional differences of cells along all three axes of bilaterian embryos, reflecting an extensive genetic regulatory network spread across the developing embryo. The resulting map of conserved expression domains represents an 'invisible anatomy'[74] that reveals clear relationships between disparate body plans, and provides a window into the organization of expression domains in the deuterostome ancestor. In this Review, we focus on the mechanisms by which these axes are formed in deuterostomes, and the patterns of transcription-factor and signalling-gene-expression domains produced along these axes.

The animal–vegetal axis and formation of endomesoderm

One of the first developmental decisions in embryogenesis is the establishment of the animal–vegetal axis. This axis sets up the formation of the three germ layers: endoderm, mesoderm and ectoderm. Ectoderm derives from the animal pole, and endomesoderm from the vegetal pole, which later divides into endoderm and mesoderm. In all three major deuterostome phyla, the formation of endomesoderm is triggered by β-catenin protein, the intracellular effector of the canonical Wnt signalling pathway. β-Catenin is stabilized preferentially in the vegetal pole of early embryos and activates genes of the endomesodermal cellular program[75,76].

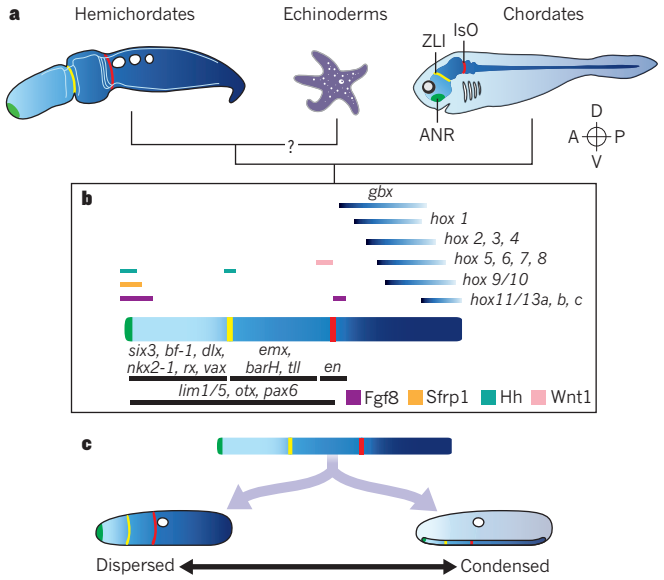


Figure 3 | A conserved molecular network for the deuterostome anteroposterior axis. **a**, Schematic representation of the distribution of ectodermal expression domains of anteroposterior (AP) transcription factors (blue gradient) and ectodermal signalling centres (green, yellow and red) in relation to the body plans of deuterostome phyla. Chordate neuroectodermal signalling centres depicted are the anterior neural ridge (ANR), zona limitans intrathalamica (ZLI) and isthmic organizer (IsO). Broad conservation of expression domains between hemichordates and chordates allows for the reconstruction of an ancestral patterning network, which is shown without any explicit inference of ancestral morphologies (**b**). Insufficient data exist from echinoderms to infer to what extent they share this conserved AP patterning network during adult patterning, although much of the anterior network is conserved in larvae[13,49]. **b**, Domain map for the conserved transcription factors and signalling ligands in relation to the AP axis[63,85,86]. **c**, Current data allow for the reconstruction of a conserved molecular coordinate system for the AP axis of the last common deuterostome ancestor, but not for the reconstruction of discrete morphologies of that ancestor, because this AP patterning network is deployed in a variety of morphological contexts, as evidenced by comparative data from hemichordates (dispersed; AP expression domains encircling the body) and chordates (condensed; AP domains largely restricted to regions near the dorsal midline). A, anterior; P, posterior; D, dorsal; V, ventral.

In hemichordates and echinoderms, knockdown of the gene that encodes β-catenin protein results in the 'animalization' of the embryo — excess ectoderm and no endomesoderm. Conversely, stabilization of the protein throughout the embryo results in 'vegetalization' of the embryo — excess endomesoderm and no ectoderm. This mechanism has also been demonstrated in protostomes from work on nemertine embryos[77], and β-catenin protein is also involved in endoderm formation in cnidarians[78], suggesting a deep eumetazoan ancestry for this process[73].

Later specification of mesoderm from the endomesoderm occurs by either of two generic mechanisms: autonomous specification by a cell's inheritance of a sequestered cytoplasmic determinant, or induction by a signal from neighbouring tissue. In all deuterostomes except ascidians, mesoderm formation occurs by induction. In vertebrates, two main signalling pathways are involved in mesoderm specification: Nodal and FGF[79]. In amphioxus, FGF signalling specifies anterior mesoderm that forms by enterocoely[42]. Similarly, in the hemichordate *S. kowalevskii*, FGF signalling induces mesoderm and enterocoely, which raises the possibility of an ancestral role of FGF in deuterostome mesoderm formation[80]. As a classic deuterostome character, a mechanistic link of enterocoely to FGF signalling would support homology of this trait, at least within the deuterostomes. In echinoderms, however, the role of FGF has yet to be fully characterized, and there is some variation in inductive cues involved in mesoderm specification: Notch–Delta signalling is important in early mesoderm specification of echinoids, but not asteroids[81]. The differences between deuterostomes in specifying endomesoderm and mesoderm preclude the definitive inference of the pathway of the deuterostome ancestor, except that β-catenin protein is required at the start, and various inductive signals are required later.

Anteroposterior axis

Although deuterostome taxa show an impressive array of morphologies, organs and cell types along the AP axis, many of the early developmental steps of axis formation are highly conserved and probably date back to the bilaterian ancestor. Wnt signalling through β-catenin has emerged as the earliest conserved determinant of AP pattern in deuterostomes. (Note that this time and place of usage of β-catenin is separate from its role in endomesoderm formation discussed earlier.) In vertebrates, Wnt proteins act as posteriorizing signals in all three germ layers, but are most analysed in CNS patterning[82,83]. Whereas Wnts are produced posteriorly, Wnt antagonists are produced anteriorly from the mesoderm of Spemann's organizer, and their interaction sets up a graded Wnt distribution prefiguring the eventual anatomical AP axis[84]. In both sea urchin larvae and the direct-developing *S. kowalevskii*, Wnt signalling is also important for establishing AP patterning[48,63,75], suggesting that generating a Wnt signalling gradient (high posteriorly, low anteriorly) is a key step in AP-axis formation in all three phyla, for both adult and larval body plans. Different intensities of Wnt signalling along the graded distribution then activate distinct genes encoding different transcription factors and signalling ligands, producing a long-lasting AP map of gene expression domains that is collinear with the Wnt distribution. The ectodermal map is strikingly similar in the identity and relative expression of the constituent regulatory genes across bilaterians[85,86].

This conserved AP map provides a novel basis for comparing body plans (Fig. 3a, b)[74]. In the most anterior regions, coexpression of genes such as *sfrp1/5*, *fgf8/17/18*, *foxG*, *retinal homeobox*, *dlx* and *nk2-1* define ectodermal territories that later form proboscis ectoderm in hemichordates and forebrain in vertebrates. Further posteriorly, expression domains of *emx*, *barH*, *dmbx* and *pax6* define the collar ectoderm of hemichordates and midbrain of vertebrates; still more posteriorly, domains of *gbx*, *engrailed*, *pax2/5/8* and the collinearly expressed Hox genes, regulate pharynx and trunk patterning of hemichordates and the hindbrain and spinal cord in vertebrates (Fig. 3a)[63,85,86]. Enteropneust Hox genes are organized as an intact cluster[87], and in both cases the posterior group Hox genes are expressed in post-anal parts of the body axis, perhaps indicating domain-level homology of these deuterostomian posterior appendages. AP map similarities even extend to three signalling centres, producing the same signals and occupying equivalent map positions, that are important for vertebrate brain patterning and for hemichordate ectodermal development at the anterior tip, proboscis–collar boundary and collar–trunk boundary (Fig. 3a,b) (for an alternative perspective see ref. 88). In hemichordates, the conserved AP map of ectodermal expression domains covers both neural and epidermal tissue, and domains encircle the body. In chordates most comparative studies have focused on the role of this network in patterning the dorsal CNS, but more recent studies demonstrate that expression of many of the genes extend ventrally into sensory neurons and epidermis, suggesting a more general role in ectodermal patterning[88].

The AP map of expression domains provides a positional criterion for evaluating morphological homologies between disparate body plans. Thus, the homology of chordate and hemichordate gill slits is supported by the observation that in both groups the first slit perforates the same region of the AP expression map, near the midbrain–hindbrain boundary in chordates and the collar–trunk boundary in enteropneusts. The map also provokes comparisons: if the hemichordate collar cord is homologous to the chordate dorsal nerve cord, it should express AP genes similar to those of the chordate midbrain. We can confidently reconstruct this AP patterning network in the ectoderm of the deuterostome ancestor, and as previously mentioned, much of the map probably dates back to the bilaterian ancestor. Indeed, more comparisons with protostomes are needed to illuminate which few domains are deuterostome-unique, for example,

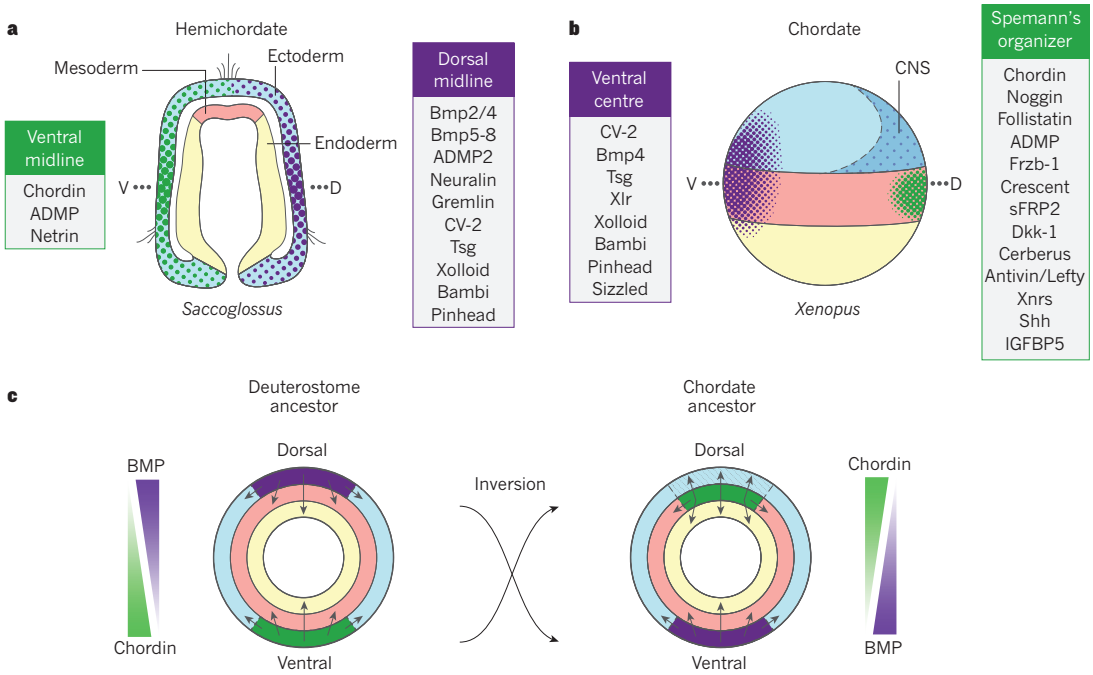


Figure 4 | Comparison of the dorsoventral patterning mechanisms of hemichordates and chordates. **a**, BMP–Chordin signalling components expressed in the dorsal and ventral midline ectoderm (blue) in the late gastrula stage of *Saccoglossus kowalevskii*. **b**, BMP–Chordin signalling components expressed either on the ventral side or dorsally in Spemann's organizer in the early gastrula of *Xenopus*. CNS, central nervous system. **c**, The inversion of dorsoventral (DV) signalling centres and the relocation of the Chordin source from the ectoderm (yellow) to mesoderm (red) were innovations in DV patterning at the base of the chordates (ancestral location shown by grey shading).

in pharyngeal ectoderm and endoderm.

The AP axial homology of chordates and hemichordates with echinoderms is far less clear[89–93]. During the development of the larvae of asteroids, echinoids and crinoids (Box 2), anterior regulatory genes are expressed throughout the anterior ectoderm[49,94], whereas posterior markers such as Hox genes are entirely absent during early patterning. In both echinoids and crinoids, it is not until adult patterning begins in the late larva that Hox genes begin expression in a collinear pattern, not in ectoderm, but in posterior coelomic mesoderm[90,95]. Some anterior markers are expressed in the oral ectoderm and tube feet of pentaradial adults, but current data are too fragmentary to make valid comparisons of adult echinoderms with other deuterostome adults. Comprehensive characterization of the patterning of echinoderm adults is badly needed to unravel the evolution of this unusual body plan.

Although the AP map is conserved across deuterostomes (and in most aspects, across bilaterians) the differentiated morphologies built on it are probably not (Fig. 3c). The morphological outcomes of development differ in each phylum because the transcription factors and signals of the conserved map activate and repress different target genes[63]. These target genes, in turn, direct the final steps of organogenesis, morphogenesis and cell-type formation.

The dorsoventral dimension

The DV axis evolved on the Pre-Cambrian stem leading to the bilaterian ancestor, and is intimately tied with the origin of bilateral symmetry. Its formation in early embryogenesis is analogous to AP axis formation. One midline of the embryo produces Bmp, and the opposite midline produces the Bmp antagonist Chordin[96]. Through complex interactions, this antagonism generates a graded distribution of Bmp across the embryo, a graded occupancy of Bmp receptors, and a corresponding graded distribution of activated Smad1/5 transcription factor in embryonic cells. This gradient of activated Smad1/5 stimulates and represses different genes encoding transcription factors and other signalling ligands, generating a long-lasting DV map of expression domains of these genes[5,97] (Fig. 4a, b).

The patterns of transcription-factor and signalling-ligand expression established along the DV direction generate the corresponding anatomical axis by driving the expression of genes for the development of different tissues and cell types in different regions. Some of the definitive tissues and cell types are remarkably conserved among bilaterians, as demonstrated by the similarities between the DV development of protostomes such as the fruit fly and the annelid *Platynereis dumerilii* and vertebrates such as *Xenopus*, mice and zebrafish[97,98]. Domains from the Chordin side of the Bmp distribution activate axial (striated) muscle development in the mesoderm and nerve-cell development in the ectoderm, especially motor neurons and interneurons that assemble into the CNS, whereas domains from the Bmp side activate heart tube and coelom development from the mesoderm and epidermis and sensory-nerve-cell development from the ectoderm[97]. The Bmp distribution patterns all three germ layers.

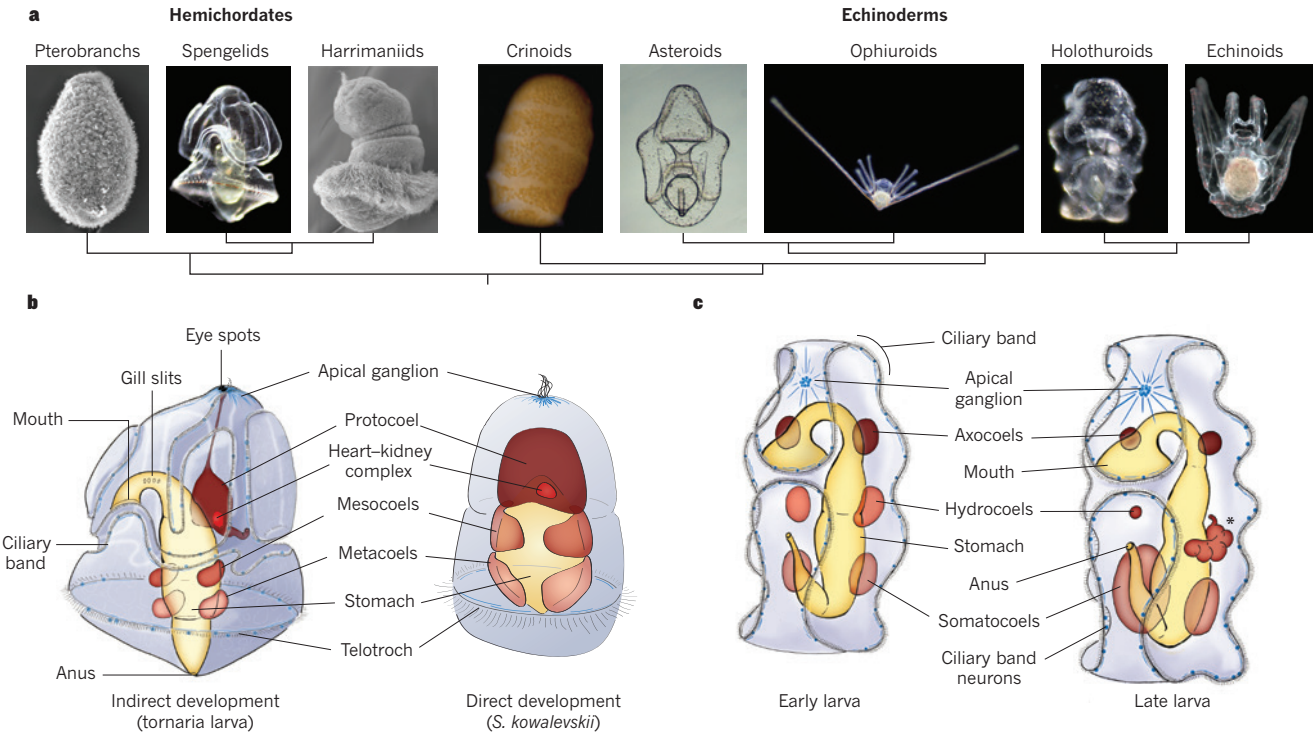
Although deuterostomes as a group inherited the basic mechanism of DV axis formation from the bilateral ancestor, there are important differences among them that can inform hypotheses of chordate origins. It is immediately apparent that the Chordin and Bmp sides of the molecular DV axis have different anatomical names in deuterostomes and protostomes. In deuterostomes, the Bmp side is called 'dorsal' and the Chordin side is 'ventral', but in *Drosophila* and other protostomes the molecular and anatomical links are reversed. By zoological convention, sides are named according to the animal's orientation to the substratum and the location of the mouth. The difference was resolved by the proposal that the chordate ancestor underwent a dorsoventral inversion of the body relative to the substratum. This transition simultaneously inverted the Bmp–Chordin axis, the domain map, and axis of anatomical differentiations[5,96]. As a final refinement the mouth was relocated to the Bmp side, whereas most protostomes (for example, *Drosophila*), and invertebrate deuterostomes, form the mouth on the Chordin side. Although seeming modest as a novelty, body inversion must be considered when discussing innovations of the chordate line.

S. kowalevskii provides an excellent example of bilaterian DV axiation, probably conserved from the deuterostome ancestor[72]. (Indirect developing hemichordates and echinoderms also exhibit Bmp–Chordin-based DV patterning, modified for larval body plans, although we cannot cover these here[9,99].) At gastrula stages, *bmp2/4* is strongly expressed on the dorsal ectodermal midline of *S. kowalevskii*, accompanied by genes for a large

BOX 2

Deuterostome larval diversity

a, Hemichordates and echinoderms include lineages that are characterized by both direct-developers, forming the adult body plan from embryogenesis in a matter of days; and by indirect-developers, first forming planktonic feeding larvae that may swim and feed for months before metamorphosing to produce a distinct adult body plan. The harrimaniid enteropneusts and pterobranchs (reproduced from ref. 49) are examples of direct-developers. **b**, A model of a two-day-old embryo of *Saccoglossus kowalevskii* contrasts with the month-old late spengelid tornaria larva. **c**, The organization of the tornaria larval body plan is very similar to the organization of the echinoderm larva represented here by a holothuroid auricularian larva. However, echinoderms have a spectacular variety of larval forms from the ophiopluteus and echinopluteus with similar elaborate skeletons to the asteroid bipinnarian and holthuroid auricularian larvae with similar convoluted ciliary bands. Many researchers have focused on the morphological and developmental similarities between the diverse ambulacrarian larval types, suggesting the existence of an ancestral 'dipleurula' (small two-sided) larval form from which ambulacrarian larval diversity arose[114]. The dipleurula ectoderm is characterized by a convoluted ciliary band used for swimming and feeding. In hemichordates, a robust additional posterior band of compound cilia, the telotroch, is purely locomotory (**a**, **b**). The nervous system is divided into two domains: an apical ganglion underlying the sensory ciliated apical organ, and neurons underlying the length of the ciliary bands (**b**, **c**). The dipleurula mesoderm is formed by enterocoely and organized into three compartments: anterior, middle and posterior. In echinoderms, the adult body plan is initiated by the left middle coelom, which expands and forms five lobes midway through larval development (**b**, asterisk in **c**). An influential theory of Garstang[108] further elaborated by a variety of authors (see Review by Holland *et al*. on page 450), proposed that the deuterostome ancestor also had a dipleurula larva, and that chordates evolved by paedomorphosis from such forms. A central tenet of this theory is that the dorsal central nervous system of chordates evolved through the dorsal migration and fusion of the lateral ciliary bands of the dipleurula larvae, and their underlying neurons[114]. More recently, this hypothesis has fallen out of favour on the basis of both phylogenetic and body-patterning data[21,24,109].



set of signal modulating proteins and other Bmp-related proteins. Conversely, *chordin* and *admp* are strongly expressed on the opposite, ventral midline (Fig. 4a). Following the Bmp distribution gradient, transcription-factor genes are activated in a DV map that generally parallels the expression of orthologous genes in *Drosophila* and vertebrates. The DV domain map and subsequent differentiated structures of the overt anatomical axis depend entirely on the Bmp distribution, as shown by the development of dorsalized embryos in the presence of excess uniform Bmp2/4 protein, and of ventralized embryos when Bmp2/4 is eliminated[72]. Tissues, organs and cell types of the three germ layers are patterned by the Bmp–Chordin distribution, including the gill slits, the mouth and the two nerve cords (Fig. 2a). In embryos dorsalized by excessive Bmp, nerve cells still form in abundance. Although this might seem contrary to chordate neural patterning in which Bmp initially represses neural development in the epidermis, it is not; the hemichordate dorsal nerve cord normally forms at the midline of high Bmp concentration, and the lateral parts of the chordate neural plate are themselves patterned by high Bmp concentrations. Overall, the hemichordate findings affirm general insights about bilaterian DV axis formation. In its body orientation, *S. kowalevskii* resembles protostomes: Bmp foretells the ventral side and Chordin the dorsal, leaving chordates as the single 'inverted' phylum (Fig. 4c). One of the key questions about chordate origins remains the evolution of the dorsal hollow nerve cord from the nervous system of a less centralized ancestor with little or no capacity for neurulation. In general, hypotheses imply that in the early embryo, the formation of neural ectoderm (prospective for motor neurons and interneurons) was increasingly repressed towards

one midline, and the neurulation process was induced along the edges of the narrowed neurectoderm territory. Thereafter Bmp exerted its neural patterning effects from the neural plate borders. This, of course, remains an area for future investigation[6,68,100].

Chordates differ from hemichordates in that Chordin and other Bmp antagonists are produced mostly in midline mesoderm, and specifically in mesoderm of Spemann's organizer, a region formed in the late blastula embryo at a location of high Nodal signalling and low Bmp signalling[101]. Organizer cells are precursors of the notochord and head mesoderm. Notochord precursors undergo extreme convergent extension by cell intercalation, forcefully repacking a cube of cells into a rod one-cell wide and lengthening the embryonic midline. Simultaneously they secrete their dorsoventral patterning molecules, neuralizing nearly half the embryo's overlying ectoderm and initiating neurulation morphogenesis. In chordates, neurogenic ectoderm produces little or no Bmp antagonist, whereas in hemichordates it is the main source. Recently, a possible notochord homologue, the axochord, was described for the polychaete annelid *P. dumerilii*; it is a midline mesodermal structure of muscle cells contained in a strong sheath into which lateral muscles attach, but there is no evidence that it is a notochord-like signalling source[102]. Rather, the midline signalling source of this protostome is presumably nearby neural ectoderm that determines, among other things, where the axochord itself develops.

Taken together, these data suggest that hemichordates are like protostomes in their dorsoventral development, whereas chordates have considerably modified the ancestral patterning mode (Fig. 4c), by adding organizer mesoderm as the Chordin source, and acquiring a large-scale neuralization response in the ectoderm. These innovations in DV axiation must be considered in any discussion of chordate origins.

Gill slits are a deuterostome innovation

The single unambiguous anatomical homology that is a clear deuterostome synapomorphy is the pharyngeal gill-slit complex[6,46,53,55,60,103]. These perforations of pharyngeal endoderm and ectoderm, ringed by beating cilia, imply that the ancestor fed by ingesting food particles carried by water flow entering the mouth and exiting the slits. The complex, which can include more than a hundred (bilaterally symmetrical) gill-slit pairs, is a major developmental and morphological modification beyond the bilaterian ancestor's pharynx, although presumably elaborated from it. Although hemichordates do not have a well-defined pharyngeal endostyle like chordates, the pharynx as a whole, and even the proboscis, probably makes endostyle-like mucociliary contributions to food trapping and conveyance to the gut[52,60]. Some of these functions may be deuterostome synapomorphies. To coordinate the functions of gill-slit-mediated water propulsion, food intake, trapping and conveyance, the pharyngeal nervous system is likely to have become modified from that of the bilateral ancestor. Given that pharyngeal innovations may represent the signature morphological, developmental and genomic innovations of deuterostomes, their development and physiology should be characterized more comprehensively.

Among extant animals, the filter-feeding lifestyle correlates with simplified body plans — radialized dorsoventral dimensions, more dispersed nervous systems, less cephalization of sensory systems, and less motility by trunk and tail axial muscles — when compared with extant food-seeking or predatory arthropods, annelids and jawed vertebrates. Such simplifications are presumably anatomical or physiological adaptations that benefit gill-slit-mediated filter feeding, and it seems plausible that evolution along the deuterostome stem involved considerable morphological modifications relative to earliest bilaterian body plans. If true, it is nonetheless apparent from AP and DV domain maps that the deuterostome ancestor suffered no concomitant loss of body-plan complexity at the molecular genetic developmental level. Rather, it shows that bilaterian domain maps are remarkably stable and can support wide-ranging morphologies, organogenesis and cytodifferentiations. An example of such modification is the muscular proboscis of hemichordates. The proboscis is used to dig and to trap food, while containing most of the conserved basic patterning elements of the vertebrate forebrain, here spread over a basiepithelial nerve plexus[63,85]. There would be no intrinsic reason for the deuterostome ancestor to preserve the morphology and differentiations of the bilaterian ancestor if it no longer lived that ancestor's lifestyle. Finally, there is some palaeontological support for a filter-feeding deuterostome ancestor. On the basis of molecular clock estimates, deuterostome phyla would have diverged in the Ediacaran period, well before the Cambrian explosion. The lack of an obvious fossil record, except for small Precambrian trace fossils and the enigmatic Ediacaran fossils, and evidence of abundant filterable food sources in the form of microbial mats and plankton, suggest that bilaterians of that time were probably small and simple filter feeders[104–106].

This interpretation of the deuterostome ancestor has important consequences for the origin of chordates. Relative to that ancestor, the chordate stem lineage achieved major developmental and morphological innovations, including the evolution of a true notochord from the archenteron roof, centralizing many morphogenetic activities of the ancestral archenteron and taking over the signalling activities of the ancestral ectoderm for both AP and DV axial patterning (by producing Bmp and Wnt antagonists), to become the centrepiece of Spemann's organizer. Concomitantly, the innovations of neural induction (neuralization) and full-length neurulation of the ectoderm generated a hollow nerve cord along the entire body length (a length now defined by the elongating notochord), rather than just the short and late collar cord neurulation of hemichordates (although this limited neurulation shows that the ancestor possessed the basic morphogenetic process and components). At some point later, dorsoventral inversion of the chordate body took place, with mouth relocation out of the neural ectoderm[107]. All of this occurred on the chordate stem, perhaps after the elimination of an ambulacrarian-type larva, to open up uninterrupted embryonic development of the adult body plan (see ref. 100 for further elaboration of this hypothesis).

Future directions

Insights into deuterostome evolution are emerging from research in developmental biology, phylogenomics, genomics and zoology. A particular focus has been the pharyngeal gill-slit complex, which is supported as an ancestral deuterostome feature by strong morphological and developmental data. The implication that the deuterostome ancestor was a filter feeder naturally draws attention to other integrated pharyngeal specializations, including endostyle-like food-trapping organs. Further study of these organs, especially in amphioxus and hemichordates, has the immediate potential to reveal clues about deuterostome and chordate origins.

One of the most important differences between hemichordates and chordates, revealed by comparative developmental studies, is the source of Bmp antagonists involved in establishing DV axial polarity in early development. These antagonists are expressed in the ectoderm of hemichordates and the mesoderm of the chordate organizer. Spemann's organizer is a key chordate developmental innovation defined by various secreted factors modulating Bmp, Nodal and Wnt signalling. A more comprehensive description of the roles of these signals and their antagonists in the patterning of hemichordate mesoderm and ectoderm will be required to devise and test hypotheses about the evolution of the chordate organizer.

Most developmental insights from hemichordates have so far come from studies of direct developing hemichordates, but a distinct larval life-history stage is probably an ancestral trait of Ambulacraria and perhaps of deuterostomes (Box 2). More comprehensive developmental studies in indirect-developing echinoderms and hemichordates, with distinct larval body plans, are needed to determine the importance of complex life cycles and the role of larvae in the early diversification of deuterostome body plans. Garstang's influential auricularian hypothesis derived the chordate body plan from an ancestral larval body plan[108], but this hypothesis has recently lost support due to revisions in chordate phylogeny and close similarities between adult rather than larval body patterning[109]. Comparative data sets on larval patterning will be key for reconstructing ancestral developmental strategies of early

deuterostomes and testing hypotheses of larval homology.

Finally, advances in genomics have begun to shed light on the gene content and chromosomal organization of invertebrate deuterostomes, including the purple sea urchin (*Strongylocentrotus purpuratus*)[110], the acorn worms *S. kowalevskii* and *Ptychodera flava*[87,111], and the crown-of-thorns sea star (*Acanthaster planci*)[112]. Given the apparent conservation of not only the pan-deuterostome axial maps but also many of the downstream factors that control organogenesis, it will be exciting to explore the gene-regulatory elements that underlie this deep conservation through a combination of comparative genomics and experimental developmental biology, revealing features of the ancestral deuterostome down to the nucleotide level. ■

Received 24 October 2014; accepted 3 February 2015.

1. Bateson, W. The ancestry of the chordata. *Q. J. Microsc. Sci.* **26,** 535–571 (1886).
2. Kowalevsky, A. Anatomie des Balanoglossus [in French]. *Mem. Acad. Imp. Sci. St Petersb.* **7,** 16 (1866).
3. Metchnikoff, V. E. Über die systematische Stellung von Balanoglossus [in German]. *Zool. Anz.* **4,** 139–157 (1881).
4. Carroll, S. B., Grenier, J. K. & Weatherbee, S. D. *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design* 2nd edn (Blackwell, 2005).
5. De Robertis, E. M. Evo-devo: variations on ancestral themes. *Cell* **132,** 185–195 (2008).
6. Brown, F. D., Prendergast, A. & Swalla, B. J. Man is but a worm: chordate origins. *Genesis* **46,** 605–613 (2008).
7. Gerhart, J., Lowe, C. & Kirschner, M. Hemichordates and the origin of chordates. *Curr. Opin. Genet. Dev.* **15,** 461–467 (2005).
8. Holland, L. Z. Chordate roots of the vertebrate nervous system: expanding the molecular toolkit. *Nature Rev. Neurosci.* **10,** 736–746 (2009).
9. Lapraz, F., Besnardeau, L. & Lepage, T. Patterning of the dorsal-ventral axis in echinoderms: insights into the evolution of the BMP-chordin signaling network. *PLoS Biol.* **7,** e1000248 (2009).
10. Satoh, N. *Developmental Genomics of Ascidians* (Wiley-Blackwell, 2014).
11. Yu, J. K. *et al*. Axial patterning in cephalochordates and the evolution of the organizer. *Nature* **445,** 613–617 (2007).
This article reports molecular evidence for a functional organizer in amphioxus, and supports the presence of this molecular patterning module at the base of the chordates.
12. Röttinger, E. & Lowe, C. J. Evolutionary crossroads in developmental biology: hemichordates. *Development* **139,** 2463–2475 (2012).
13. Angerer, L. M., Yaguchi, S., Angerer, R. C. & Burke, R. D. The evolution of nervous system patterning: insights from sea urchin development. *Development* **138,** 3613–3623 (2011).
14. Peter, I. S. & Davidson, E. H. A gene regulatory network controlling the embryonic specification of endoderm. *Nature* **474,** 635–639 (2011).
15. Swalla, B. J. & Smith, A. B. Deciphering deuterostome phylogeny: molecular, morphological and palaeontological perspectives. *Phil. Trans. R. Soc. Lond. B* **363,** 1557–1568 (2008).
16. Haeckel, E. H. P. A. *Anthropogenie: oder, Entwickelungsgeschichte des Menschen* [in German] (Wilhelm Engelmann, 1874).
17. Kowalevsky, A. Weitere Studien über die Entwicklung der einfachen Ascidien [in German]. *Arch. Mikr. Anat.* **7,** 101–130 (1866).
18. Darwin, C. *The Descent of Man, and Selection in Relation to Sex* (D. Appleton and company, 1871).
19. Grobben, K. Die systematische einteilung des tierreiches [in German]. *Verh. der Zool.-Bot. Ges. Wien.* **58,** 491–511 (1908).
20. Martin-Duran, J. M., Janssen, R., Wennberg, S., Budd, G. E. & Hejnol, A. Deuterostomic development in the protostome *Priapulus caudatus*. *Curr. Biol.* **22,** 2161–2166 (2012).
21. Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439,** 965–968 (2006).
The authors of this paper provide conclusive molecular evidence for the sister relationship between tunicates and vertebrates and the basal position of cephalochordates.
22. Dunn, C. W. *et al*. Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* **452,** 745–749 (2008).
23. Wada, H. & Satoh, N. Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. *Proc. Natl Acad. Sci. USA* **91,** 1801–1804 (1994).
24. Blair, J. E. & Hedges, S. B. Molecular phylogeny and divergence times of deuterostome animals. *Mol. Biol. Evol.* **22,** 2275–2284 (2005).
25. Field, K. G. *et al*. Molecular phylogeny of the animal kingdom. *Science* **239,** 748–753 (1988).
26. Halanych, K. M. The phylogenetic position of the pterobranch hemichordates based on 18S rDNA sequence data. *Mol. Phylogenet. Evol.* **4,** 72–76 (1995).
27. Philippe, H. *et al*. Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. *Nature* **470,** 255–258 (2011).
28. Hejnol, A. *et al*. Assessing the root of bilaterian animals with scalable phylogenomic methods. *Proc. Biol. Sci.* **276,** 4261–4270 (2009).
29. Gee, H. *Before the Backbone* (Chapman & Hall, 1996).
30. Stach, T. Chordate phylogeny and evolution: a not so simple three-taxon problem. *J. Zool.* **276,** 117–141 (2008).
31. Lu, T. M., Luo, Y. J. & Yu, J. K. BMP and Delta/Notch signaling control the development of amphioxus epidermal sensory neurons: insights into the evolution of the peripheral sensory system. *Development* **139,** 2020–2030 (2012).
32. Escriva, H., Holland, N. D., Gronemeyer, H., Laudet, V. & Holland, L. Z. The retinoic acid signaling pathway regulates anterior/posterior patterning in the nerve cord and pharynx of amphioxus, a chordate lacking neural crest. *Development* **129,** 2905–2916 (2002).
33. Onai, T. *et al*. Retinoic acid and Wnt/β-catenin have complementary roles in anterior/posterior patterning embryos of the basal chordate amphioxus. *Dev. Biol.* **332,** 223–233 (2009).
34. Meulemans, D. & Bronner-Fraser, M. Gene-regulatory interactions in neural crest evolution and development. *Dev. Cell* **7,** 291–299 (2004).
35. Shimeld, S. M. The evolution of the hedgehog gene family in chordates: insights from amphioxus hedgehog. *Dev. Genes Evol.* **209,** 40–47 (1999).
36. Holland, L. Z. *et al*. Evolution of bilaterian central nervous systems: a single origin? *EvoDevo* **4,** 27 (2013).
37. Irimia, M. *et al*. Conserved developmental expression of *Fezf* in chordates and *Drosophila* and the origin of the *Zona Limitans Intrathalamica* (ZLI) brain organizer. *EvoDevo* **1,** 7 (2010).
38. Abedin, M. & King, N. The premetazoan ancestry of cadherins. *Science* **319,** 946–948 (2008).
39. Conklin, E. G. The embryology of amphioxus. *J. Morphol.* **54,** 69–151 (1932).
40. Gomez, C. *et al*. Control of segment number in vertebrate embryos. *Nature* **454,** 335–339 (2008).
41. Beaster-Jones, L. *et al*. Expression of somite segmentation genes in amphioxus: a clock without a wavefront? *Dev. Genes Evol.* **218,** 599–611 (2008).
42. Bertrand, S. *et al*. Amphioxus FGF signaling predicts the acquisition of vertebrate morphological traits. *Proc. Natl Acad. Sci. USA* **108,** 9160–9165 (2011).
43. Kozmik, Z. *et al*. Pax-Six-Eya-Dach network during amphioxus development: conservation *in vitro* but context specificity *in vivo*. *Dev. Biol.* **306,** 143–159 (2007).
44. Mazet, F., Amemiya, C. T. & Shimeld, S. M. An ancient Fox gene cluster in bilaterian animals. *Curr. Biol.* **16,** R314–316 (2006).
45. Jandzik, D. *et al*. Evolution of the new vertebrate head by co-option of an ancient chordate skeletal tissue. *Nature* **518,** 534–537 (2015).
46. Rychel, A. L. & Swalla, B. J. Development and evolution of chordate cartilage. *J. Exp. Zool. B Mol. Dev. Evol.* **308,** 325–335 (2007).
47. Wright, G. M., Keeley, F. W. & Robson, P. The unusual cartilaginous tissues of jawless craniates, cephalochordates and invertebrates. *Cell Tissue Res.* **304,** 165–174 (2001).
48. Range, R. C., Angerer, R. C. & Angerer, L. M. Integration of canonical and noncanonical Wnt signaling pathways patterns the neuroectoderm along the anterior–posterior axis of sea urchin embryos. *PLoS Biol.* **11,** e1001467 (2013).
49. Yankura, K. A., Martik, M. L., Jennings, C. K. & Hinman, V. F. Uncoupling of complex regulatory patterning during evolution of larval development in echinoderms. *BMC Biol.* **8,** 143 (2010).
50. Sato, A., Bishop, J. D. & Holland, P. W. Developmental biology of pterobranch hemichordates: history and perspectives. *Genesis* **46,** 587–591 (2008).
51. Dominguez, P., Jacobson, A. G. & Jefferies, R. P. Paired gill slits in a fossil with a calcite skeleton. *Nature* **417,** 841–844 (2002).
52. Gonzalez, P. & Cameron, C. B. The gill slits and pre-oral ciliary organ of *Protoglossus* (Hemichordata: Enteropneusta) are filter-feeding structures. *Biol. J. Linn. Soc.* **98,** 898–906 (2009).
53. Gillis, J. A., Fritzenwanker, J. H. & Lowe, C. J. A stem-deuterostome origin of the vertebrate pharyngeal transcriptional network. *Proc. Biol. Sci.* **279,** 237–246 (2012).
This report shows extensive patterning similarities between the development of chordate and enteropneust gills, further supporting morphological homology.
54. Ogasawara, M., Wada, H., Peters, H. & Satoh, N. Developmental expression of *Pax1/9* genes in urochordate and hemichordate gills: insight into function and evolution of the pharyngeal epithelium. *Development* **126,** 2539–2550 (1999).
55. Fritzenwanker, J. H., Gerhart, J., Freeman, R. M. Jr & Lowe, C. J. The Fox/Forkhead transcription factor family of the hemichordate *Saccoglossus kowalevskii*. *EvoDevo* **5,** 17 (2014).
56. Balser, E. J. & Ruppert, E. E. Structure, ultrastructure, and function of the preoral heart-kidney in *Saccoglossus kowalevskii* (Hemichordata, Enteropneusta) including new data on the stomochord. *Acta Zool.* **71,** 235–249 (1990).
57. Miyamoto, N. & Wada, H. Hemichordate neurulation and the origin of the neural tube. *Nature Commun.* **4,** 2713 (2013).
This manuscript demonstrates similar mediolateral patterning mechanisms between the hemichordate collar cord and chordate dorsal cord.
58. Hyman, L. H. *The Invertebrates* 1st edn (McGraw-Hill, 1959).
59. Luttrell, S., Konikoff, C., Byrne, A., Bengtsson, B. & Swalla, B. J. Ptychoderid hemichordate neurulation without a notochord. *Integr. Comp. Biol.* **52,** 829–834 (2012).
60. Ruppert, E. E. Key characters uniting hemichordates and chordates: homologies or homoplasies? *Can. J. Zool.* **83,** 8–23 (2005).
61. Satoh, N. *et al*. On a possible evolutionary link of the stomochord of hemichordates to pharyngeal organs of chordates. *Genesis* **52,** 925–934 (2014).
62. De Robertis, E. M. & Kuroda, H. Dorso-ventral patterning and neural induction in *Xenopus* embryos. *Annu. Rev. Cell Dev. Biol.* **20,** 285–308 (2004).

63. Pani, A. M. *et al*. Ancient deuterostome origins of vertebrate brain signalling centres. *Nature* **483,** 289–294 (2012).
This paper presents evidence that ectodermal signalling centres thought to have been uniquely associated with the evolution of vertebrate brains are present in hemichordates as part of a conserved ancient deuterostome patterning network.
64. Bullock, T. H. The anatomical organization of the nervous system of enteropneusta. *Q. J. Microsc. Sci*. **86,** 55–111 (1945).
65. Kaul, S. & Stach, T. Ontogeny of the collar cord: neurulation in the hemichordate *Saccoglossus kowalevskii*. *J. Morphol*. **271,** 1240–1259 (2010).
66. Knight-Jones, E. On the nervous system of *Saccoglossus cambriensis* (Enteropneusta). *Phil. Trans. R. Soc. Lond. B* **236,** 315–354 (1952).
67. Morgan, T. Development of *Balanoglossus*. *J. Morphol.* **9,** 1–86 (1894).
68. Benito-Gutierrez, E. & Arendt, D. CNS evolution: new insight from the mud. *Curr. Biol*. **19,** R640–642 (2009).
69. Nomaksteinsky, M. *et al*. Centralization of the deuterostome nervous system predates chordates. *Curr. Biol*. **19,** 1264–1269 (2009).
This paper shows clear molecular evidence for the presence of cell bodies in the dorsal nerve cord of enteropneusts and proposes the deep ancestry of a CNS in the deuterostomes.
70. Bullock, T. H. The functional organisation of the nervous system of the Enteropneusta. *Biol. Bull*. **79,** 91–113 (1940).
71. Cameron, C. B. & Mackie, G. O. Conduction pathways in the nervous system of *Saccoglossus* sp. (Enteropneusta). *Can. J. Zool*. **74,** 15–19 (1996).
72. Lowe, C. J. *et al*. Dorsoventral patterning in hemichordates: insights into early chordate evolution. *PLoS Biol*. **4,** e291 (2006).
This manuscript demonstrates the role of BMP signalling in the formation of the DV axis of the enteropneust adult body plan.
73. Martindale, M. Q. & Hejnol, A. A developmental perspective: changes in the position of the blastopore during bilaterian evolution. *Dev. Cell* **17,** 162–174 (2009).
74. Slack, J. M., Holland, P. W. & Graham, C. F. The zootype and the phylotypic stage. *Nature* **361,** 490–492 (1993).
75. Darras, S., Gerhart, J., Terasaki, M., Kirschner, M. & Lowe, C. J. β-Catenin specifies the endomesoderm and defines the posterior organizer of the hemichordate *Saccoglossus kowalevskii*. *Development* **138,** 959–970 (2011).
76. Wikramanayake, A. H., Huang, L. & Klein, W. H. β-Catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. *Proc. Natl Acad. Sci. USA* **95,** 9343–9348 (1998).
77. Henry, J. Q., Perry, K. J., Wever, J., Seaver, E. & Martindale, M. Q. β-Catenin is required for the establishment of vegetal embryonic fates in the nemertean, *Cerebratulus lacteus*. *Dev. Biol*. **317,** 368–379 (2008).
78. Wikramanayake, A. H. *et al*. An ancient role for nuclear β-catenin in the evolution of axial polarity and germ layer segregation. *Nature* **426,** 446–450 (2003).
79. Kimelman, D. Mesoderm induction: from caps to chips. *Nature Rev. Genet*. **7,** 360–372 (2006).
80. Green, S. A., Norris, R. P., Terasaki, M. & Lowe, C. J. FGF signaling induces mesoderm in the hemichordate *Saccoglossus kowalevskii*. *Development* **140,** 1024–1033 (2013).
81. Hinman, V. F. & Davidson, E. H. Evolutionary plasticity of developmental gene regulatory network architecture. *Proc. Natl Acad. Sci. USA* **104,** 19404–19409 (2007).
82. Hikasa, H. & Sokol, S. Y. Wnt signaling in vertebrate axis specification. *Cold Spring Harb. Perspect. Biol.* **5,** a007955 (2013).
83. Petersen, C. P. & Reddien, P. W. Wnt signaling and the polarity of the primary body axis. *Cell* **139,** 1056–1068 (2009).
84. Kiecker, C. & Niehrs, C. A morphogen gradient of Wnt/β-catenin signalling regulates anteroposterior neural patterning in *Xenopus*. *Development* **128,** 4189–4201 (2001).
85. Lowe, C. J. *et al*. Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* **113,** 853–865 (2003).
This paper provides evidence for a conserved transcriptional gene regulatory network between hemichordates and chordates despite large organizational differences in their basic body plans.
86. Aronowicz, J. & Lowe, C. J. Hox gene expression in the hemichordate *Saccoglossus kowalevskii* and the evolution of deuterostome nervous systems. *Integr. Comp. Biol*. **46,** 890–901 (2006).
87. Freeman, R. *et al*. Identical genomic organization of two hemichordate hox clusters. *Curr. Biol*. **22,** 2053–2058 (2012).
88. Holland, L. Z. *et al*. Evolution of bilaterian central nervous systems: a single origin? *EvoDevo* **4,** 27 (2013).
89. David, B. & Mooi, R. How Hox genes can shed light on the place of echinoderms among the deuterostomes. *EvoDevo* **5,** 22 (2014).
90. Hara, Y. *et al*. Expression patterns of Hox genes in larvae of the sea lily *Metacrinus rotundus*. *Dev. Genes Evol*. **216,** 797–809 (2006).
91. Morris, V. B. & Byrne, M. Oral-aboral identity displayed in the expression of HpHox3 and HpHox11/13 in the adult rudiment of the sea urchin *Holopneustes purpurescens*. *Dev. Genes Evol*. **224,** 1–11 (2014).
92. Lacalli, T. Echinoderm conundrums: Hox genes, heterochrony, and an excess of mouths. *Evodevo* **5,** 46 (2014).
93. Peterson, K. J., Arenas-Mena, C. & Davidson, E. H. The A/P axis in echinoderm ontogeny and evolution: evidence from fossils and molecules. *Evol. Dev*. **2,** 93–101 (2000).
94. Omori, A., Akasaka, K., Kurokawa, D. & Amemiya, S. Gene expression analysis of Six3, Pax6, and Otx in the early development of the stalked crinoid *Metacrinus rotundus*. *Gene Expr. Patterns* **11,** 48–56 (2011).
95. Arenas-Mena, C., Cameron, A. R. & Davidson, E. H. Spatial expression of Hox cluster genes in the ontogeny of a sea urchin. *Development* **127,** 4631–4643 (2000).
96. De Robertis, E. M. & Sasai, Y. A common plan for dorsoventral patterning in Bilateria. *Nature* **380,** 37–40 (1996).
97. Mizutani, C. M. & Bier, E. EvoD/Vo: the origins of BMP signalling in the neuroectoderm. *Nature Rev. Genet*. **9,** 663–677 (2008).
98. Denes, A. S. *et al*. Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* **129,** 277–288 (2007).
99. Röttinger, E. & Martindale, M. Q. Ventralization of an indirect developing hemichordate by $NiCl_2$ suggests a conserved mechanism of dorso-ventral (D/V) patterning in Ambulacraria (hemichordates and echinoderms). *Dev. Biol.* **354,** 173–190 (2011).
100. Satoh, N. An aboral-dorsalization hypothesis for chordate origin. *Genesis* **46,** 614–622 (2008).
101. Gerhart, J. Evolution of the organizer and the chordate body plan. *Int. J. Dev. Biol*. **45,** 133–153 (2001).
102. Lauri, A. *et al*. Development of the annelid axochord: insights into notochord evolution. *Science* **345,** 1365–1368 (2014).
103. Cameron, C. B., Garey, J. R. & Swalla, B. J. Evolution of the chordate body plan: new insights from phylogenetic analyses of deuterostome phyla. *Proc. Natl Acad. Sci. USA* **97,** 4469–4474 (2000).
This manuscript proposes that the extant enteropneust, rather than pterobranch, adult body plan may best represent ancestral deuterostome characters.
104. Erwin, D. H. *et al*. The Cambrian conundrum: early divergence and later ecological success in the early history of animals. *Science* **334,** 1091–1097 (2011).
105. Erwin, D. H. & Valentine, J. *The Cambrian Explosion: the Construction of Animal Biodiversity* (Roberts and Company, 2013).
106. Peterson, K. J. & Butterfield, N. J. Origin of the Eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proc. Natl Acad. Sci. USA* **102,** 9547–9552 (2005).
107. Lacalli, T. C. The emergence of the chordate body plan: some puzzles and problems. *Acta Zool*. **91,** 4–10 (2010).
108. Garstang, W. The morphology of the Tunicata. *Q. J. Microsc. Sci*. **72,** 51–187 (1928).
109. Lacalli, T. C. Protochordate body plan and the evolutionary role of larvae: old controversies resolved? *Can. J. Zool*. **83,** 216–224 (2005).
110. Sea Urchin Genome Sequencing Consortium. The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science* **314,** 941–952 (2006).
111. Freeman, R. M. Jr *et al*. cDNA sequences for transcription factors and signaling proteins of the hemichordate *Saccoglossus kowalevskii*: efficacy of the expressed sequence tag (EST) approach for evolutionary and developmental studies of a new organism. *Biol. Bull*. **214,** 284–302 (2008).
112. Baughman, K. W. *et al*. Genomic organization of Hox and ParaHox clusters in the echinoderm, *Acanthaster planci*. *Genesis* **52,** 952–958 (2014).
113. Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439,** 965–968 (2006).
114. Nielsen, C. Origin of the chordate central nervous system — and the origin of chordates. *Dev. Genes Evol*. **209,** 198–205 (1999).
115. Gudo, M. & Syed, T. 100 years of Deuterostomia (Grobben, 1908): Cladogenetic and anagenetic relations within the notoneuralia domain. http://arxiv.org/abs/0811.2189 (2008).
116. Jefferies, R. P. S. *The Ancestry of the Vertebrates* (Cambridge Univ. Press, 1986).
117. Swalla, B. J. Building divergent body plans with similar genetic pathways. *Heredity* **97,** 235–243 (2006).
118. Bone, Q. The central nervous system in amphioxus. *J. Comp. Neurol*. **115,** 27–51 (1960).
119. Wicht, H. & Lacalli, T. C. The nervous system of amphioxus: structure, development, and evolutionary significance. *Can. J. Zool.* **83,** 122–150 (2005).
120. Cannon, J. T. *et al*. Phylogenomic resolution of the hemichordate and echinoderm clade. *Current Biol*. **24,** 2827–2832 (2014).
121. Cameron, C. B. Particle retention and flow in the pharynx of the enteropneust worm *Harrimania planktophilus*: the filter-feeding pharynx may have evolved before the chordates. *Biol. Bull*. **202,** 192–200 (2002).
122. Telford, M. J. *et al*. Phylogenomic analysis of echinoderm class relationships supports Asterozoa. *Proc. Biol. Sci*. **281,** 20140479 (2014).

Acknowledgments We thank K. Bertsche (http://wanderingfalcon.com), for the scientific illustrations, C. Patton and J. Watanabe for photography of invertebrate micrographs, K. Halanych and J. Cannon for providing pterobranch images, and J. Fritzenwanker for the German translation of Grobben, and helpful discussions. We apologize to authors whose work we were unable to cite due to space limitations, and thank M. Kirschner, A. Pani, T. Lacalli, N. Satoh and N. Holland for discussions that helped formulate these ideas. Support for this work was awarded to C.J.L from NASA (NNX13AI68G) and NSF (1258169), to D.M.M. from NSF (IOS1257040). D.S.R. is supported by the Okinawa Institute of Science and Technology and the US National Institutes of Heath through grant R01 GM086321. Work at the Joint Genome Institute is supported by the Office of Science of the US Department of Energy under contract number DE-AC02-05CH11231.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this paper at go.nature.com/wmiwjq. Correspondence should be addressed to C.J.L. (clowe@stanford.edu).

REVIEW

doi:10.1038/nature14435

A new heart for a new head in vertebrate cardiopharyngeal evolution

Rui Diogo[1], Robert G. Kelly[2], Lionel Christiaen[3], Michael Levine[4], Janine M. Ziermann[1], Julia L. Molnar[1], Drew M. Noden[5] & Eldad Tzahor[6]

It has been more than 30 years since the publication of the new head hypothesis, which proposed that the vertebrate head is an evolutionary novelty resulting from the emergence of neural crest and cranial placodes. Neural crest generates the skull and associated connective tissues, whereas placodes produce sensory organs. However, neither crest nor placodes produce head muscles, which are a crucial component of the complex vertebrate head. We discuss emerging evidence for a surprising link between the evolution of head muscles and chambered hearts — both systems arise from a common pool of mesoderm progenitor cells within the cardiopharyngeal field of vertebrate embryos. We consider the origin of this field in non-vertebrate chordates and its evolution in vertebrates.

In their influential 1983 paper, Gans and Northcutt[1] proposed that early vertebrates evolved from invertebrates principally through innovations in the head. These include the muscularization of the ventrolateral mesoderm, or hypomere, to form branchiomeric muscles and the emergence of two novel ectodermal structures: the neurogenic placodes and the neural crest. Neural crest cells produce most of the cartilage, bone, dentine and other connective tissues of the vertebrate head, whereas the placodes give rise to the sensory neurons that are essential for the formation of vertebrates' complex sensory systems[2–4]. The new head hypothesis proposed that these evolutionary innovations were associated with a shift from passive filter-feeding to active predation. Increased sensory capabilities and a muscularized pharynx arguably permitted more efficient prey detection and capture, as well as higher rates of respiratory gas exchange, which accompany the predatory lifestyle. This major behavioural and ecological transition also coincided with the emergence of a chambered heart, which presumably allowed for the increased growth and metabolism that was demanded by active predation. However, the new head hypothesis was primarily concerned with derivatives of neural crest and placodes, which are better represented in the fossil record than soft tissues such as muscles[5,6]. In this Review, we provide an up-to-date multidisciplinary discussion of the origin and evolution of vertebrate head muscles, taking into account surprising new evidence for shared developmental origins of several head muscles and the heart, and the ancient (pre-vertebrate) origin of this association.

The emerging concept of the cardiopharyngeal field

The cardiopharyngeal field (CPF) is a developmental domain that gives rise to the heart and branchiomeric muscles (Box 1 and Figs 1, 2). The amniote heart is made up of cardiomyocytes derived from two adjacent progenitor cell populations in the early embryo[7]. Early differentiating cardiac progenitor cells of the first heart field (FHF) give rise to the linear heart tube and later form the left ventricle and parts of the atria[8,9]. Subsequently, second-heart-field (SHF) progenitors, located in pharyngeal mesoderm, produce cardiac muscle tissue (myocardium) of the outflow tract, right ventricle and parts of the atria[10–12] (Fig. 2). The SHF can be divided into anterior and posterior progenitor cell populations that contribute to the arterial and venous poles of the heart, respectively[8]. Cells from pharyngeal mesoderm can form either cardiac or skeletal muscles, depending on signals from adjacent pharyngeal endoderm, surface ectoderm and neural crest cells[9,13–16]. The latter have important roles in regulating the development of the CPF — they are required for the deployment of SHF-derived cells to the heart's arterial pole, and neural-crest-derived mesenchyme patterns branchiomeric muscle formation and gives rise to associated fascia and tendons[17–19].

A suite of regulatory factors integrates the intercellular signals that coordinate the formation of cardiac and branchiomeric muscles from a common pool of mesodermal progenitor cells. Within the CPF there is considerable overlap in the expression of genes that encode cardiogenic regulatory factors (for example, *Isl1* (also known as *Islet1*) and *Nkx2-5*) and those that specify head muscles (for example, *Tbx1*, *Tcf21* (also known as capsulin), *Msc* (also known as *MyoR*) and *Pitx2*)[13,15,20]. Importantly, many of the intercellular signalling pathways and transcription factors that control branchiomeric myogenesis upstream of the MyoD family of myogenic determination factors differ fundamentally from those operating in the trunk[21,22]. Here we focus on Isl1, Nkx2-5 and Tbx1. The LIM-homeodomain protein Isl1 is required in a broad subset of cardiovascular progenitor cells in mouse embryos[23] and it is expressed in pharyngeal mesoderm, including the pharyngeal arches and SHF. Isl1$^+$ progenitor cells substantially contribute to the heart and branchiomeric muscles, but not to hypobranchial (for example, tongue) or extraocular (eye) muscles[13,24]. Expression and functional studies indicate that Isl1 delays differentiation of branchiomeric muscles[13,24]; Isl1 thus marks a subset of CPF cells and plays an important part in the development of distinct cardiovascular and skeletal muscle progenitors[24]. The cardiac transcription factor Nkx2-5 regulates proliferation in the SHF and acts with Isl1 to modulate SHF progenitor-specific gene expression[25–27]. *Tbx1* is required within the CPF for both heart and head muscle development, and is the major candidate gene for the congenital condition DiGeorge syndrome (or 22q11.2 deletion syndrome), which is characterized by a spectrum of cardiovascular defects and craniofacial anomalies. Like Isl1, Tbx1 has a crucial and conserved role in extending the heart's arterial pole by promoting proliferation and delaying differentiation of SHF cells[28–31]. Tbx1 is also required for activation of branchiomeric myogenesis and may directly regulate the myogenic determination gene *MyoD*[32–34].

[1]Department of Anatomy, Howard University College of Medicine, Washington DC 20059, USA. [2]Aix Marseille Université, Centre National de la Recherche Scientifique, Institut de Biologie du Développement de Marseille UMR 7288, 13288 Marseille, France. [3]Center for Developmental Genetics, Department of Biology, New York University, New York 10003, USA. [4]Department of Molecular and Cell Biology, University of California at Berkeley, California 94720, USA. [5]Department of Biomedical Sciences, College of Veterinary Medicine , Cornell University, Ithaca, New York 14853, USA. [6]Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100, Israel.

Tbx1 acts upstream of the LIM-homeodomain protein Lhx2 within an intricate regulatory network that specifies cardiopharyngeal progenitors. Genetic ablation of these factors, alone or in combination, results in cardiac and head muscle defects; including DiGeorge syndrome phenotypes[35]. Thus, evolutionarily conserved regulatory factors maintain a pool of cardiopharyngeal progenitor cells for SHF-specific cardiogenesis and branchiomeric myogenesis.

Confirmation that multipotent progenitor cells give rise to branchiomeric skeletal muscles and SHF-derived regions of the heart comes from retrospective clonal analyses in mice, a method for analysing cell lineage in intact embryos[36]. These experiments demonstrated the existence of a series of common cardiopharyngeal progenitors along the anteroposterior axis that contribute to heart-tube growth and branchiomeric muscle morphogenesis. Interestingly, comparative anatomists suggested decades ago that branchiomeric muscles are related to muscles derived from the 'visceral' mesoderm (for example, of the heart and anterior gut)[37,38], a view supported by the recent genetic and developmental studies reviewed here. Moreover, mouse clonal analyses revealed relationships between specific regions of the heart and subsets of branchiomeric muscles that go beyond the predictions of early comparative anatomists. SHF-derived regions of the heart, for example, are developmentally more closely related to branchiomeric muscles than to FHF-derived regions of the heart[7,36]. In support of such a grouping, the cardiac lineages contributing to the FHF and SHF have been shown to diverge before expression of *Mesp1* during early gastrulation[39,40]. Taken together, recent findings provide a new paradigm for exploring the collinear emergence of cardiac chambers and branchiomeric muscles that underlies the early evolution and diverse origins of the vertebrate head[9,21,22,41,42].

Origins and diversity of cardiopharyngeal structures

The heads of mammals, including humans, contain more than 60 muscles[43], which control eye movements and allow food uptake, respiration, and facial and vocal communication[44–46]. Strikingly, the human head includes at least six different groups of muscles with distinct developmental origins and evolutionary histories[35,37,44] (Fig. 1). Full recognition and detailed knowledge of this heterogeneity has enormous basic science and clinical implications because long accepted anatomy concepts, mainly based on adult function and physiology (for example, skeletal compared with cardiac muscles) do not correspond to the true developmental and evolutionary origins of body structures. Even the conventional classification of head muscle groups based on topographical relations masks the true heterogeneity of muscle origins and progenitor fates (for example, molecular profiling of early determinative signalling molecules and transcription factors reveals almost as much heterogeneity within each group — such as, branchial, extraocular and tongue — as between them[43]).

Comparative anatomical studies identified homologues of many amniote branchiomeric muscles in gnathostome (jawed) fish such as sharks, suggesting that they have ancient origins[47,48] (Fig. 3). Cyclostomes (hagfish and lampreys[49–52]) lack some of these muscles (for example, the cucullaris group), but like some chondrichthyans (Selachii and Holocephali) they possess an additional, seventh group of head muscles: epibranchial muscles, which are derived from anterior somites[53]. Thus, extraocular, branchiomeric, and both hypobranchial and epibranchial somite-derived muscles were integral parts of the heterogenous head musculature of early vertebrates[54–57] (Fig. 3). Moreover, lamprey embryos express homologues of *Isl1*, *Nkx2-5* and *Tbx1* in seemingly overlapping anterior and ventral mesodermal domains[58–61], comparable with the patterns of their homologues in the amniote CPF. Interestingly, the emergence of heterogeneous head-muscle groups at the base of vertebrates coincided with the emergence of chambered hearts[62,63] (Fig. 3). This intriguing correlation suggests that the two innovations are linked by their common developmental origin in the CPF.

Studies indicate that specific branchiomeric muscles were crucial for evolutionary innovations among vertebrates, such as the emergence of the tetrapod neck. The amniote neck muscles trapezius and sternocleidomastoideus (Fig. 1) derive from the cucullaris, a muscle that probably appeared in early gnathostomes and was found in fossil placoderms[5,6,48,64,65]. Among extant gnathostomes, some of the anatomical and developmental characteristics of the cucullaris are shared with branchiomeric and somite-derived limb, epibranchial and hypobranchial muscles[57,66,67]. Most available data, however, indicate that the cucullaris is a branchiomeric muscle derived from the posterior-most pharyngeal arches, as suggested by Edgeworth[22,68–71]. Like other branchiomeric muscles, in most gnathostomes the cucullaris is attached to neural-crest-derived tendinous and skeletal elements[38,64,65,70,72]. Furthermore, *Tbx1* is active in core branchiomeric muscles (for example, the first and second arch muscles) and in the cucullaris-derived trapezius, whereas *Pax3* is required in the somites for limb, diaphragm, tongue, infrahyoid and trunk-muscle formation, but not for trapezius formation[22,73]. These findings may also support Gegenbaur's hypothesis that the pectoral appendage, to which the cucullaris and its derivatives usually attach, probably originated as an integral part of the head[74,75]. Thus, the evolutionary history of the cucullaris-related muscles illustrates the roles that branchiomeric muscles had in fostering anatomical and functional innovations during vertebrate evolution. Future studies are needed to investigate whether the emergence of the cucullaris at the base of gnathostomes coincided with cardiovascular innovations and, if so, whether this muscle also shares a common origin with a specific heart region (Fig. 1).

A urochordate cardiopharyngeal ontogenetic motif

Recent phylogenetic studies place the urochordates — not the cephalochordates (for example, amphioxus) — as the sister group of the

BOX 1
Glossary

- **Branchiomeric muscles.** Muscles formed from progenitor cells found in the pharyngeal arches. In vertebrates, they comprise the mandibular (first arch muscles, such as jaw muscles), hyoid (second arch muscles, such as the facial expression muscles of mammals) and branchial (from more posterior arches, including muscles of the larynx and pharynx, and the cucullaris-derived neck muscles trapezius and sternocleidomastoideus, in amniotes) muscles.
- **Pharyngeal (or branchial) arches.** Bilateral swellings on either side of the pharynx comprising outer (ectodermal) and inner (endodermal) epithelia, neural-crest-derived mesenchyme and a mesodermal core.
- **First heart field.** Population of early differentiating cardiac progenitor cells that arise in anterior lateral mesoderm and give rise to the linear heart tube and, later, to the left ventricle and parts of the atria.
- **Second heart field.** Population of late differentiating cardiac progenitors that contribute to the developing heart after the linear heart tube stage to give rise to myocardium of the right ventricle and outflow tract, and to inflow tract myocardium, including parts of the atria.
- **Cardiopharyngeal field.** Includes anterior lateral mesoderm of the first heart field plus contiguous pharyngeal mesoderm that gives rise to second-heart-field-derived regions of the heart and branchiomeric muscles.
- **Cardiopharyngeal ontogenetic motif.** Lineage-specific progression through cardiopharyngeal progenitor cell identities, with conserved clonal relationships between first heart, second heart and pharyngeal muscle precursors characterized by specific gene expression and regulatory activities.
- **Pharyngeal mesoderm.** Cranial mesoderm associated with the forming foregut or pharynx that populates pharyngeal arches and contributes to second-heart-field-derived regions of the heart and branchiomeric muscles.

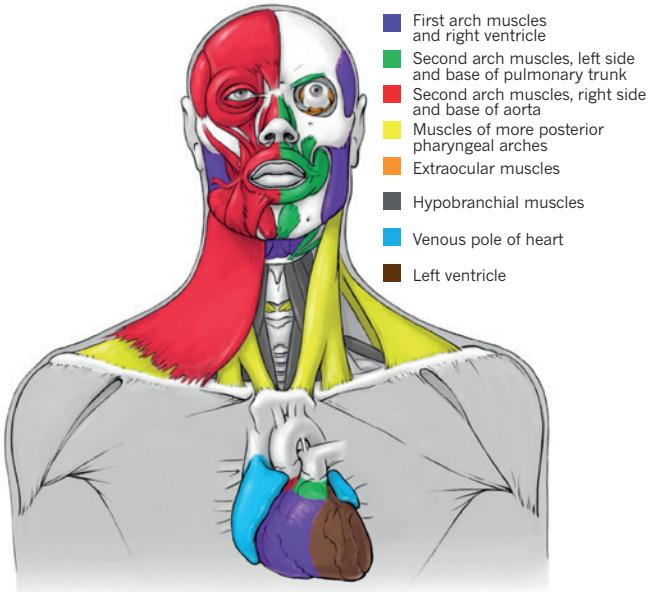


Figure 1 | The striking heterogeneity of the human head and heart musculature. The head includes at least six different muscle groups, all arising from the cardiopharyngeal field and being branchiomeric, except the hypobranchial and perhaps the extraocular muscles. On the left side of the body (right part of figure) the facial expression muscles have been removed to show the masticatory muscles. The six groups are: first/mandibular arch muscles, including cells clonally related to the right ventricle; left second/hyoid arch muscles related to myocardium at the base of the pulmonary trunk; right second/hyoid arch muscles, related to myocardium at the base of the aorta; muscles of the most posterior pharyngeal arches, including muscles of the pharynx and larynx and the cucullaris-derived neck muscles trapezius and sternocleidomastoideus; extraocular muscles, which are often not considered to be branchiomeric, but according to classic embryological studies and recent retrospective clonal analyses in mice contain cells related to those of the branchiomeric mandibular muscles; and hypobranchial muscles, including tongue and infrahyoid muscles that derive from somites and migrate into the head and neck[36,38,70].

vertebrates[76,77]. On the basis of these results, urochordates provide important insights for our understanding of the origin of vertebrates' evolutionary innovations, particularly from molecular and developmental perspectives. For instance, the new head hypothesis proposed that the emergence of branchiomeric muscles occurred during the transitions that led to the origin of vertebrates, and was associated with a shift from 'passive' filtration to more active feeding modes[1,4,78,79] and the emergence of crest- and placode-derived sensory organs. However, recent studies have identified neural-crest-like cells, placodes and a CPF in tadpole-like larvae of the ascidian *Ciona intestinalis*, a model urochordate (Figs 2, 4). The pan-placodal regulatory gene *Six1/2* is expressed in a crescent of cells straddling the anterior-most region of the developing neural tube in *C. intestinalis* embryos, comparable with the sites of origin of cranial placodes in the fate maps of vertebrates[80–82]. Ectodermal thickenings derived from this domain express placodal regulatory genes, including *Six3/6*, *Pitx* and *Eya*. For example, the atrial siphon placode shares extensive similarities with the vertebrate otic placode[3,80,81] (Fig. 4), whereas the stomodeum (the oral siphon primordium) expresses regulatory genes implicated in the specification of the vertebrate olfactory and adenohypophyseal placodes, including *Six*, *Eya* and the anterior placode markers *Pitx*[83–85] and *Dlx*. These new findings argue for homologies between urochordate siphon primordia and vertebrate placodes and suggest that; although certain placodes (profundal, maxillomandibular, epibranchial and lens) evolved by diversification within the vertebrate lineage[3], others (adenohypophyseal, olfactory and otic) appeared before the separation of vertebrates and urochordates (Figs 3, 4).

Ascidians and other urochordates possess a surprisingly sophisticated beating heart (Figs 2, 4), which shares several features with vertebrate hearts, including localized pacemakers that drive a regular, rhythmic beat. The ascidian heart is derived from two Mesp$^+$ cells in early embryos. These produce four trunk ventral cells, which express homologues of *Nkx2-5*, *Gata4, 5* and *6* and *Hand*, and migrate towards the pharyngeal endoderm[86–92]. They subsequently divide asymmetrically to produce medial heart precursors and secondary trunk ventral cells that divide again to produce second heart precursors and atrial siphon muscle precursors, which migrate towards the atrial siphon placode[93–95] (Figs 2, 4). Thus, trunk ventral cells are multipotent cardiopharyngeal progenitors that produce bona fide heart and pharyngeal muscles, following a clonal pattern evocative of that seen in mice (Fig. 2). Gene-expression profiling data are also consistent with the idea that the trunk ventral cells are homologous to the vertebrate cardiopharyngeal progenitors: trunk ventral cells express *Nk4*, the homologue of *Nkx2-5*, and secondary trunk ventral cells also express *Tbx1/10*, which is active in vertebrate pharyngeal mesoderm. Furthermore, the regulatory network governing interactions among the cardiopharyngeal specification genes seems to be highly conserved in ascidians and vertebrates. For example, cross-repressive interactions between Tbx1/10 and Nk4/Nkx2-5 delineate atrial siphon muscles and heart, respectively[95]. *Isl* is also expressed in the CPF, although there are differences from the precise expression profile seen in vertebrates, where Isl1 is thought to delay muscle differentiation[24]. It is nonetheless striking that all of the identified molecular determinants of the vertebrate SHF are expressed in ascidian trunk ventral cells.

There are additional parallels between the CPFs of ascidians and vertebrates in the regulatory circuitry underlying the differentiation of specialized muscles (Fig. 2). COE/Ebf functions downstream of Tbx1/10 and upstream of both Mrf/MyoD and Notch signalling to promote either early muscle differentiation or maintain undifferentiated precursors that produce most later atrial siphon and longitudinal muscles[93,96] (Fig. 2). Atrial siphon muscle precursors also associate with the Dlx$^+$ atrial siphon placodes to form a ring of cells underlying the rosette-shaped placode in *C. intestinalis* swimming larvae[80,81,93,97]. These events parallel the migration of vertebrate branchiomeric muscle precursors into pharyngeal arches, their association with Dlx$^+$ cranial neural crest cells, and the maintenance and growth of a pool of undifferentiated progenitor cells[24,98]. It is noteworthy that the ascidian FHF and SHF are each initially composed of four cells that independently arise from one of four multipotent cardiopharyngeal progenitors following a sequence of conserved regulatory interactions onto a stereotyped clonal pattern, producing FHF precursors and more closely related SHF and pharyngeal muscle precursors[95]. We refer to this clonal sequence of cell divisions, gene expression and cell-fate choices as a cardiopharyngeal ontogenetic motif[95] (Fig. 2).

Chordate origins of branchiomeric muscles

Studies using cephalochordates further probed the early chordate origins of branchiomeric-like pharyngeal muscles (Figs 3, 4). In the cephalochordate amphioxus, the larval mouth and unpaired primary gills develop five groups of orobranchial muscles[99,100]. This musculature is anatomically reminiscent of the vertebrate branchiomeric muscles, and disappears through apoptosis during metamorphosis to give way to adult oral, velar and pterygial muscles[99] (Fig. 4), which are even more similar to vertebrate adult branchiomeric muscles. The oral and velar muscles, in particular, share anatomical similarities with the oral and velar muscles of lampreys and hagfish (Fig. 4), although the pterygial muscles have a branchiomeric-like innervation pattern[99]. Gans[79] recognized this latter point and noted that this could mean that the branchiomeric muscles evolved before the last common ancestor (LCA) of vertebrates, as suggested by earlier authors[22], but contrary to the original new head hypothesis[1]. Vestigial muscles appear transiently with secondary gill formation in amphioxus, providing additional evidence that bilateral muscular gills and a segmental pattern of branchiomeric muscles were already present in the LCA of extant chordates[22].

Molecular studies suggest that the amphioxus homologues of *Tbx1*, *Nkx2-5* and *Isl1* are expressed in overlapping mesodermal domains in the pharyngeal region[101–103]. This domain includes cells that also express the

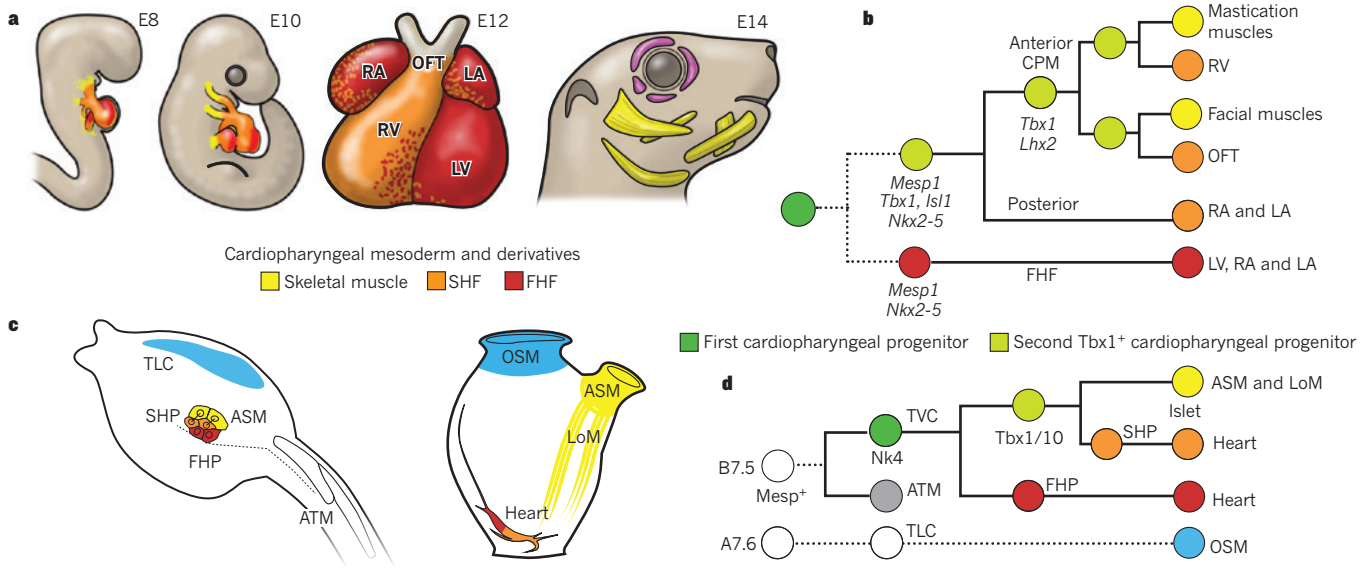


Figure 2 | An evolutionarily conserved cardiopharyngeal ontogenetic motif. a, Mouse embryos at embryonic days (E)8 and 10, the four-chambered mouse heart at E12, and the mouse head at E14. First heart field (FHF)-derived regions of heart (left ventricle (LV) and atria) are in red; second heart field (SHF)-derived regions of heart (right ventricle (RV), left atrium (LA), right atrium (RA) and outflow tract (OFT)) are in orange; branchiomeric skeletal muscles are in yellow; extraocular muscles are in purple. **b**, Lineage tree depicting the origins of cardiac compartments and branchiomeric muscles in mice. All cells derive from common pan-cardiopharyngeal progenitors (dark green) that produce the FHF, precursors of the left ventricle and atria, and the second Tbx1$^+$ cardiopharyngeal progenitors (light green). Broken lines indicate that the early common FHF and SHF progenitor remains to be identified in mice. In anterior cardiopharyngeal mesoderm (CPM), progenitor cells activate Lhx2, self-renew and produce the SHF-derived RV and OFT, and first and second arch branchiomeric muscles (including muscles of mastication and facial expression). **c**, Cardiopharyngeal precursors in *Ciona intestinalis* hatching larva (left) and their derivatives in the metamorphosed juvenile (right). The first heart precursors (FHP) (red) and second heart precursors (SHP) (orange) contribute to the heart (red and orange mix), whereas atrial siphon muscle precursors (ASM, yellow) form atrial siphon and longitudinal muscles (LoM, yellow). Oral siphon muscles (OSM, blue) derive from a heterogenous larval population of trunk lateral cells (TLC, blue). ATM, anterior tail muscles. CPM is bilaterally symmetrical around the midline (dotted line). **d**, Lineage tree depicting clonal relationships and gene activities deployed in *C. intestinalis* cardiopharyngeal precursors. All cells derive from Mesp$^+$ B7.5 blastomeres, which produce ATM (grey, see also left panel of **c**) and trunk ventral cells (TVC, dark green). The latter pan-cardiopharyngeal progenitors express Nk4 and divide asymmetrically to produce the FHP (red) and second TVCs, the Tbx1/10$^+$ second cardiopharyngeal progenitors (second TVC, light green disk). The latter divide again asymmetrically to produce SHP (orange) and the precursors of ASM and LoM, which upregulate Islet. The OSM arise from A7.6-derived trunk lateral cells (TLC, light blue).

vertebrate cardiac markers *Hand* and *Tbx20* (refs 59, 104) and is thought to produce the branchial artery, a possible — but controversial — homologue of the heart with diffuse contractility[105]. These observations raise the possibility that the LCA of extant chordates had a CPF. However, contrary to urochordates and vertebrates, cephalochordates have a rather diffuse heart-like vasculature and their branchial muscles seem to develop independently of *Ebf* and *Mrf* homologues[94,106,107]. Amphioxus *Mrf* homologues seem to be expressed exclusively in somites, overlapping with the *Pax3/7* homologue[106,108], but also with the *Tbx1* homologue[102], suggesting the presence of distinct Tbx1$^+$, Pax3/7$^+$, Mrf$^+$ somitic and Tbx1$^+$, Pax3/7$^-$, Mrf$^-$ pharyngeal mesodermal domains in ancient chordates.

Branchiomeric-like muscles, such as the cephalochordate oral, velar and pterygial muscles (Fig. 4), thus probably predate the origin of a CPF as defined in urochordates and vertebrates (Fig. 3). Comparative anatomical studies suggest that the pterygial and orovelar muscles of adult amphioxus probably correspond to the atrial and oral siphon muscles of urochordates, respectively (Fig. 4). Remarkably, the ascidian oral siphon muscles (Fig. 4), which control mouth movements in post-metamorphic animals, do not derive from cardiopharyngeal progenitors[93,109,110] (Fig. 2). This is in contrast with the anterior oral muscles controlling mouth movements and in particular jaw opening (first (mandibular) arch muscles) in gnathostomes, which are CPF derivatives (Fig. 2). Comparative studies of basal chordates, including that of the fossil *Haikouella*, suggested that their pharyngeal arch series started with the second (hyoid) arch and that only during early vertebrate evolution did parts of the anterior mesoderm become incorporated into the pharyngeal series by forming a new, *Hox*-independent first arch[111,112]. Therefore, it is possible that the incorporation of the more anterior (first) arch in this series during vertebrate evolution was accompanied by integration of the associated oral and velar muscles into the CPF. This evolutionary scenario implies that the amphioxus orovelar muscles and urochordate oral siphon muscles may be homologous to the cyclostome orovelar muscles and gnathostome mandibular muscles, which could potentially explain why these muscles are derived from the CPF only in vertebrates.

Bilaterian roots of the cardiopharyngeal network

We have argued that the presence of a CPF, with dual cardiac and skeletal myogenic capacity, is probably a synapomorphy of olfactores (a derived feature shared by urochordates and vertebrates; Figs 2, 3). This argument raises the question: do the developmental, cellular and/or molecular units that form the CPF network of olfactores have even deeper evolutionary origins? Ambulacraria (echinoderms and hemichordates) is the sister group of chordates (Fig. 3). Hemichordates possess well-defined serial gill slits and a heart–kidney complex located in the anterior-most body part (proboscis)[113]. Serially arranged pharyngeal gill openings have associated muscles in enteropneust-type hemichordates, but this musculature seems to be developmentally, anatomically and histologically distinct from the chordate branchiomeric musculature[99]. Moreover, the *Tbx1* homologue of *Saccoglossus kowalevskii*, an enteropneust hemichordate, is not expressed in the mesodermal core of the pharyngeal pouches[114], suggesting that *Tbx1* expression in pharyngeal mesoderm is a chordate synapomorphy. Further studies of ambulacrarians will test this hypothesis.

Among non-deuterostome animals, nematodes lack a heart and a defined circulatory system, but possess pharyngeal muscles that contract rhythmically, exhibit electrical activity similar to mammalian cardiomyocytes, and require *ceh-22*, the homologue of *Nkx2-5* (refs 9, 21, 22, 41, 42, 115). Flies lack anatomical structures that are comparable with the chordate pharyngeal apparatus, but the *Drosophila* homologues of *Tbx1*,

Nkx2-5, *Isl*, *Ebf* and *Mrf/MyoD* variably contribute to visceral, larval and adult skeletal and/or heart muscle specification[116–121]. The diversity of myogenic networks driving muscle identity and differentiation in flies is reminiscent of the heterogeneity of myogenic origins and programs operating in the vertebrate head. Furthermore, visceral and dorsal larval muscles in *Drosophila* develop from mesoderm in proximity to the dorsal vessel or fly heart. It is therefore conceivable that many features of the CPF gene regulatory network predate the advent of chordates and, moreover, that this regulatory circuitry preceded the emergence of the well-studied myogenic hierarchies controlling vertebrate somitic muscle development.

Evolvable cardiopharyngeal units

Here, we summarize our arguments for the origins and diversification of the CPF (Fig. 3). Filter-feeding early chordates, endowed with serial gill slits inherited from deuterostome ancestors, already had gill-associated branchiomeric, or at least branchiomeric-like, muscles (Fig. 4). A well-defined CPF then probably appeared in the olfactores. Ancestral vertebrates uncoupled myogenic specification and differentiation, thus increasing the population of cardiopharyngeal progenitors. This facilitated the emergence of cardiac chambers by progressive addition of progenitor cells to the growing heart tube during development. It also allowed for the expansion and diversification of branchiomeric muscles, contributing to increased muscularization of the pharyngeal apparatus that was essential for the transition to a predatory lifestyle. The latter was made possible by olfactores' ancestral association between branchiomeric muscles and Dlx^+ ectoderm cells. Elaboration of this interaction permitted coevolution of the branchiomeric musculature with the newly formed neural crest-derived craniofacial skeleton, linking the novel neural-crest-derived skeletal patterns with distinct branchiomeric muscles.

We propose that the heart and atrial siphon muscle gene network seen in the urochordate *C. intestinalis* illustrates the basic ontogenetic motif underlying the specification of the vertebrate CPF[95], and suggest three ways in which this blueprint was modified to produce the vast diversity of cardiopharyngeal patterns in vertebrates: the ontogenetic motif could be deployed in multiple independent embryonic progenitors; any given progenitor could self-renew, thus being transiently amplified, before generating distinct heart, in contrast with branchiomeric, muscle precursors and any given cell could migrate and/or be passively displaced and resume cardiopharyngeal development in different locations on receipt of appropriate signals. In contrast to their ascidian counterparts, vertebrate $Tbx1^+$ and $Isl1^+$ cardiopharyngeal progenitors remain in an elusive niche in which they self-renew to produce SHF-derived heart precursors. During pharyngeal morphogenesis, these emerge sequentially to produce right ventricular and outflow tract cardiomyocytes. Conceivably, multiple independent cardiopharyngeal lineages developing in series may contribute to divergent cardiac and branchiomeric myogenic cell fates along the anterior–posterior pharyngeal mesoderm of vertebrates. This hypothesis is consistent with the observation that subsets of cardiac and branchiomeric muscles are more closely related to each other than to other heart and head muscles (Fig. 1)[36,122,123]. Future experiments will determine whether anteroposterior patterning of the CPF precedes segmentation of the pharyngeal region during arch morphogenesis.

General remarks and future directions

The CPF is a new paradigm to be reckoned with, and should take centre stage along with neural crest and cranial placodes when considering the origin of the vertebrate head. Importantly, novel insights from comparative, phylogenomic and developmental genetics studies have uncovered the deep evolutionary origins of the CPF, branchiomeric muscles, placodes and neural crest cells. Like vertebrates, urochordates have a CPF that gives rise to the FHF, SHF and branchiomeric muscles; moreover, apart from their neural-crest-like cells and placodes, at least some pelagic urochordates have highly developed brains[124]. Data obtained after Gans and Northcutt's new head hypothesis thus call into question the clear distinction between vertebrates and other animals, and show that the 'new' head arose instead by elaboration and modification of existing tissues, cell populations and gene networks through evolutionary 'tinkering'. This revelation supports the proposal[125] that the conventional view of vertebrates evolving from brainless ascidian-like filter-feeders through a progressive increase in complexity and emergence of several *de novo* structures, with no evolutionary losses or reversions, is an oversimplification. These data also emphasize the heterogeneity and complex developmental and evolutionary history of vertebrate hearts and heads, blurring the interface between head and trunk, extraocular and branchiomeric, and skeletal and

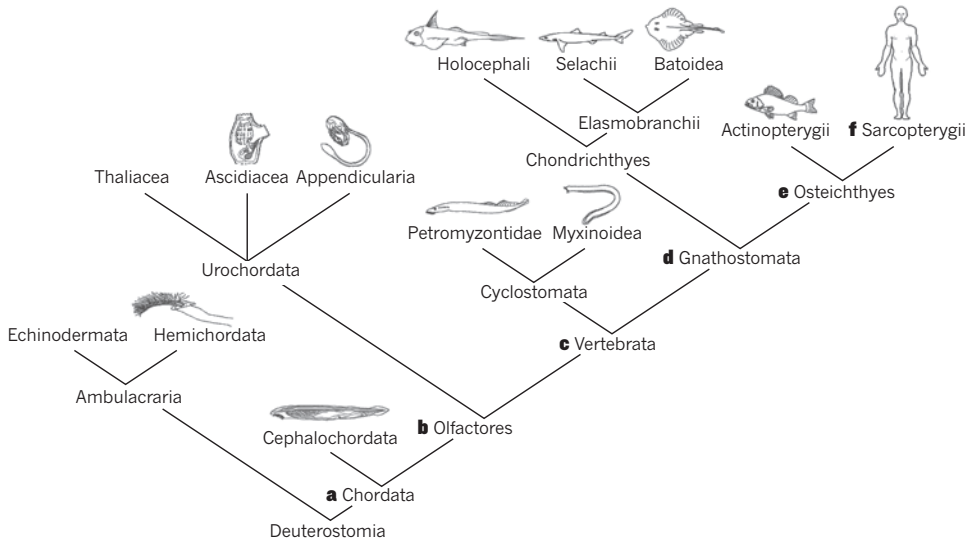


Figure 3 | Some of the synapomorphies of the Chordata and its subgroups, according to our own data and review of the literature. **a**, Somites and branchiomeric muscles. **b**, Placodes, neural-crest-like cells and cardiopharyngeal field (CPF) (although within invertebrates, conclusive evidence for these features was only reported in urochordates, some of these features may have been already present in the last common ancestor of extant chordates) giving rise to first- and second-heart-field-derived parts of the heart and to branchiomeric muscles (possibly not all of them, that is, inclusion of oral/velar muscles into CPF might have occurred during vertebrate evolution). **c**, Skull, cardiac chambers, and differentiation of epibranchial and hypobranchial somitic muscles. **d**, Jaws and differentiation between hypaxial and epaxial somitic musculature; paired appendages and fin muscles; origin of the branchiomeric muscle cucullaris. **e**, Loss of epibranchial muscles; cucullaris divided into levatores arcuum branchialium (going to pharyngeal arches) and protractor pectoralis (going to pectoral girdle), an exaptation that later allowed the emergence of the tetrapod neck. **f**, Within sarcopterygians, the protractor pectoralis gave rise to the amniote neck muscles trapezius and sternocleidomastoideus.

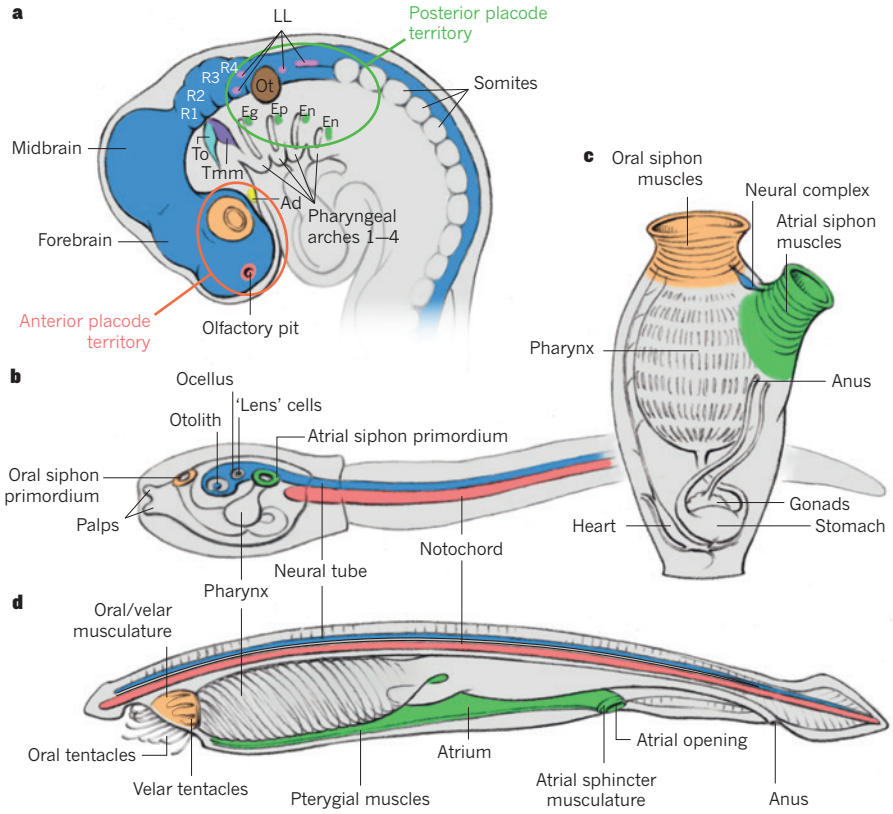


Figure 4 | Homology hypotheses of placodes and branchiomeric muscles within chordates. **a**, Location of ectodermal placodes in the vertebrate head according to Graham and Shimeld's[3] hypothesis (anterior to the left): olfactory placode or pit (red) at the tip of the forebrain; lens placodes (orange) form posteriorly as part of eye; adenohypophyseal placode (Ad, yellow) lies ventrally to forebrain; trigeminal placodes form alongside the anterior hindbrain at the levels of rhombomeres 1 and 2 (R1 and R2), the anterior one being the ophthalmic placode (To, light blue) and the posterior one the maxillomandibular placode (Tmm, purple); otic placode (Ot, brown) forms opposite the central domain of hindbrain; lateral line placodes (LL, pink) form anteriorly and posteriorly to otic placode; epibranchial placodes (green) — geniculate (Eg), petrosal (Ep) and nodose (En) — form as part of pharyngeal series. Forebrain, midbrain and R1–4, and neural tube are shown in dark blue. **b**, Urochordate tadpole-like larva (anterior to the left). The notochord is in red and two siphon primordia are in green and orange, with putative relationships to the anterior and posterior placode territories shown in **a**. **c**, Adult urochordate showing siphon primordia after metamorphosis. **d**, Adult cephalochordate showing the urochordate–cephalochordate muscle homology hypotheses proposed in the present Review. Figures based on images from refs 3, 22, 105.

cardiac myogenesis. Adult postcranial structures, including the heart and part of the neck musculature, include cells derived from the CPF (Fig. 1); reciprocally, cephalic structures such as the tongue and infrahyoid muscles arise from somitic primordia located in the trunk. The discovery of the CPF therefore provides a more complete, and complex, view of the origin and early evolution of the vertebrate head.

However, many questions remain. For example, how is the multipotency of branchiomeric and cardiac myocyte progenitor cells encoded in the CPF, and is there a defined molecular common niche in which these multipotent progenitor cells arise? How, and during what stages, are progenitor cell populations that give rise to different regions of the heart and head muscles specified in pharyngeal mesoderm? Recognition of the CPF also sets the stage for future discoveries in human medicine (Fig. 1). An important question is why many myopathies preferentially affect a specific subset of muscles, and whether these aetiologies are linked to the disparate embryonic histories of these muscles. As already noted, the clinical features of DiGeorge syndrome — one of the most common human congenital syndromes — include cardiovascular and craniofacial birth defects, highlighting the frequent link between these defects owing to their anatomical proximity during early embryogenesis and overlapping progenitor populations[9,21,42]. Therefore, the studies and data discussed here open promising new directions for biomedical research and the advancement of public health. For instance, future meta-analyses may reveal pathological relationships between specific branchiomeric muscles and regional congenital heart defects. The field of evolutionary developmental biology has progressed remarkably over the three decades since the new head hypothesis was published. With the recent revolutionary discoveries and more exciting work already begun, the field is poised to move ahead anew.

Note added in proof: A paper has been published while the current Review was in press reporting the identification of a third group of bilateral common heart and skeletal muscle progenitor cells within the murine CPM. Using retrospective lineage analysis, cucullaris-derived neck muscles, the trapezius and sternocleidomastoid, were shown to be clonally related to myocardium at the venous pole of the heart, derived from the posterior SHF. These findings reinforce the hypothesis of a branchiomeric origin of these neck muscles (F. Lescroart *et al.* Clonal analysis reveals a common origin between nonsomite-derived neck muscles and heart myocardium. *Proc. Natl Acad. Sci. USA* **112,** 1446–1451; 2015). ■

Received 30 August; accepted 25 November 2014.

1. Gans, C. & Northcutt, R. G. Neural crest and the origin of vertebrates: a new head. *Science* **220,** 268–273 (1983).
This highly influential paper argued that the evolution of head structures derived from neural crest and cranial placodes had a crucial role in the transition to early vertebrates.
2. Patthey, C., Schlosser, G. & Shimeld, S. M. The evolutionary history of vertebrate cranial placodes — I: cell type evolution. *Dev. Biol.* **389,** 82–97 (2014).
3. Graham, A. & Shimeld, S. M. The origin and evolution of the ectodermal placodes. *J. Anat.* **222,** 32–40 (2013).
4. Northcutt, R. G. The new head hypothesis revisited. *J. Exp. Zool. B Mol. Dev. Evol.* **304B,** 274–297 (2005).
5. Kuratani, S. Evolution. A muscular perspective on vertebrate evolution. *Science* **341,** 139–140 (2013).
6. Trinajstic, K. *et al.* Fossil musculature of the most primitive jawed vertebrates. *Science* **341,** 160–164 (2013).
7. Meilhac, S. M., Esner, M., Kelly, R. G., Nicolas, J. F. & Buckingham, M. E. The clonal origin of myocardial cells in different regions of the embryonic mouse heart. *Dev. Cell* **6,** 685–698 (2004).
8. Kelly, R. G. The second heart field. *Curr. Top. Dev. Biol.* **100,** 33–65 (2012).
9. Tzahor, E. & Evans, S. M. Pharyngeal mesoderm development during embryogenesis: implications for both heart and head myogenesis. *Cardiovasc. Res.* **91,** 196–202 (2011).
10. Kelly, R. G., Brown, N. A. & Buckingham, M. E. The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm. *Dev. Cell* **1,** 435–440 (2001).
Discovery of the mammalian SHF, demonstrating that myocardium at the arterial pole of the heart originates in adjacent pharyngeal mesoderm.
11. Mjaatvedt, C. H. *et al.* The outflow tract of the heart is recruited from a novel heart-forming field. *Dev. Biol.* **238,** 97–109 (2001).
12. Waldo, K. L. *et al.* Conotruncal myocardium arises from a secondary heart field. *Development* **128,** 3179–3188 (2001).
13. Nathan, E. *et al.* The contribution of Islet1-expressing splanchnic mesoderm cells to distinct branchiomeric muscles reveals significant heterogeneity in head muscle development. *Development* **135,** 647–657 (2008).
This article provides a definition of the contribution of pharyngeal mesoderm to branchiomeric muscles in both chick and mouse embryos.
14. Mesbah, K. *et al.* Identification of a *Tbx1/Tbx2/Tbx3* genetic pathway governing pharyngeal and arterial pole morphogenesis. *Hum. Mol. Genet.* **21,** 1217–1229 (2012).
15. Tirosh-Finkel, L., Elhanany, H., Rinon, A. & Tzahor, E. Mesoderm progenitor cells

of common origin contribute to the head musculature and the cardiac outflow tract. *Development* **133,** 1943–1953 (2006).
This article demonstrates, using fate-mapping and experimental manipulation in the avian embryo, that cranial mesoderm gives rise both to head muscles and outflow tract myocardium.
16. Tzahor, E. & Lassar, A. B. Wnt signals from the neural tube block ectopic cardiogenesis. *Genes Dev.* **15,** 255–260 (2001).
17. Noden, D. M. & Trainor, P. A. Relations and interactions between cranial mesoderm and neural crest populations. *J. Anat.* **207,** 575–601 (2005).
18. Hutson, M. R. & Kirby, M. L. Neural crest and cardiovascular development: a 20-year perspective. *Birth Defects Res. C Embryo Today* **69,** 2–13 (2003).
19. Rinon, A. *et al*. Cranial neural crest cells regulate head muscle patterning and differentiation during vertebrate embryogenesis. *Development* **134,** 3065–3075 (2007).
20. Bothe, I. & Dietrich, S. The molecular setup of the avian head mesoderm and its implication for craniofacial myogenesis. *Dev. Dynam.* **235,** 2845–2860 (2006).
21. Grifone, R. & Kelly, R. G. Heartening news for head muscle development. *Trends Genet.* **23,** 365–369 (2007).
22. Sambasivan, R., Kuratani, S. & Tajbakhsh, S. An eye on the head: the development and evolution of craniofacial muscles. *Development* **138,** 2401–2415 (2011).
23. Cai, C. L. *et al*. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev. Cell* **5,** 877–889 (2003).
24. Harel, I. *et al*. Distinct origins and genetic programs of head muscle satellite cells. *Dev. Cell* **16,** 822–832 (2009).
This article demonstrates the diversity of lineages constituting craniofacial skeletal muscles and their associated satellite cells using a series of Cre lines to genetically trace trunk and cranial myogenic progenitor cells, leading to an Isl1-lineage-based definition of CPF-derived craniofacial muscles.
25. Dodou, E., Verzi, M. P., Anderson, J. P., Xu, S. M. & Black, B. L. Mef2c is a direct transcriptional target of ISL1 and GATA factors in the anterior heart field during mouse embryonic development. *Development* **131,** 3931–3942 (2004).
26. Watanabe, Y. *et al*. Fibroblast growth factor 10 gene regulation in the second heart field by Tbx1, Nkx2–5, and Islet1 reveals a genetic switch for down-regulation in the myocardium. *Proc. Natl Acad. Sci. USA* **109,** 18273–18280 (2012).
27. Prall, O. W. *et al*. An Nkx2–5/Bmp2/Smad1 negative feedback loop controls heart progenitor specification and proliferation. *Cell* **128,** 947–959 (2007).
28. Scambler, P. J. 22q11 deletion syndrome: a role for TBX1 in pharyngeal and cardiovascular development. *Pediatr. Cardiol.* **31,** 378–390 (2010).
29. Liao, J. *et al*. Identification of downstream genetic pathways of Tbx1 in the second heart field. *Dev. Biol.* **316,** 524–537 (2008).
30. Chen, L., Fulcoli, F. G., Tang, S. & Baldini, A. Tbx1 regulates proliferation and differentiation of multipotent heart progenitors. *Circ. Res.* **105,** 842–851 (2009).
31. Hami, D., Grimes, A. C., Tsai, H. J. & Kirby, M. L. Zebrafish cardiac development requires a conserved secondary heart field. *Development* **138,** 2389–2398 (2011).
32. Kelly, R. G., Jerome-Majewska, L. A. & Papaioannou, V. E. The del22q11.2 candidate gene *Tbx1* regulates branchiomeric myogenesis. *Hum. Mol. Genet.* **13,** 2829–2840 (2004).
This paper reports the genetic identification of *Tbx1* as a regulator of craniofacial myogenesis in mice, supporting the existence of distinct upstream regulatory hierarchies controlling head and trunk myogenesis.
33. Kong, P. *et al*. Tbx1 is required autonomously for cell survival and fate in the pharyngeal core mesoderm to form the muscles of mastication. *Hum. Mol. Genet.* **23,** 4215–4231 (2014).
34. Castellanos, R., Xie, Q., Zheng, D., Cvekl, A. & Morrow, B. E. Mammalian TBX1 preferentially binds and regulates downstream targets via a tandem T-site repeat. *PLoS ONE* **9,** e95151 (2014).
35. Harel, I. *et al*. Pharyngeal mesoderm regulatory network controls cardiac and head muscle morphogenesis. *Proc. Natl Acad. Sci. USA* **109,** 18839–18844 (2012).
36. Lescroart, F. *et al*. Clonal analysis reveals common lineage relationships between head muscles and second heart field derivatives in the mouse embryo. *Development* **137,** 3269–3279 (2010).
This retrospective lineage analysis provides evidence for the existence of common progenitor cells in the mouse embryo that give rise to myocardium of the right ventricle and first-arch-derived muscles, and to the arterial pole of the heart and second-arch-derived muscles.
37. Romer, A. S. & Parson, T. S. *The Vertebrate Body* (Saunder's College Publishing, 1977).
38. Diogo, R. & Abdala, V. *Muscles of Vertebrates: Comparative Anatomy, Evolution, Homologies and Development* (CRC, 2010).
This monograph provides an overview on the comparative anatomy, evolution and homologies of the head and limb muscles in all major extant vertebrate groups with special focus on the developmental and evolutionary history of the muscles of *Homo sapiens*.
39. Devine, W. P., Wythe, J. D., George, M., Koshiba-Takeuchi, K. & Bruneau, B. G. Early patterning and specification of cardiac progenitors in gastrulating mesoderm. *eLife* **3,** e03848 (2014).
40. Lescroart, F. *et al*. Early lineage restriction in temporally distinct populations of Mesp1 progenitors during mammalian heart development. *Nature Cell Biol.* **16,** 829–840 (2014).
41. Olson, E. N. Gene regulatory networks in the evolution and development of the heart. *Science* **313,** 1922–1927 (2006).
42. Tzahor, E. Heart and craniofacial muscle development: a new developmental theme of distinct myogenic fields. *Dev. Biol.* **327,** 273–279 (2009).
43. Diogo, R. & Wood, B. A. *Comparative Anatomy and Phylogeny of Primate Muscles and Human Evolution* (CRC, 2012).
44. Wachtler, F. & Jacob, M. Origin and development of the cranial skeletal muscles. *Bibl. Anat.* **1986,** 24–46 (1986).
45. Noden, D. M. The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. *Am. J. Anat.* **168,** 257–276 (1983).
46. Noden, D. M. & Francis-West, P. The differentiation and morphogenesis of craniofacial muscles. *Dev. Dynam.* **235,** 1194–1218 (2006).
47. Diogo, R., Hinits, Y. & Hughes, S. M. Development of mandibular, hyoid and hypobranchial muscles in the zebrafish: homologies and evolution of these muscles within bony fishes and tetrapods. *BMC Dev. Biol.* **8,** 24 (2008).
48. Diogo, R., Abdala, V., Lonergan, N. & Wood, B. A. From fish to modern humans — comparative anatomy, homologies and evolution of the head and neck musculature. *J. Anat.* **213,** 391–424 (2008).
49. Kuraku, S., Hoshiyama, D., Katoh, K., Suga, H. & Miyata, T. Monophyly of lampreys and hagfishes supported by nuclear DNA-coded genes. *J. Mol. Evol.* **49,** 729–735 (1999).
50. Delarbre, C., Gallut, C., Barriel, V., Janvier, P. & Gachelin, G. Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. *Mol. Phylogenet. Evol.* **22,** 184–192 (2002).
51. Delarbre, C. *et al*. The complete nucleotide sequence of the mitochondrial DNA of the agnathan *Lampetra fluviatilis*: bearings on the phylogeny of cyclostomes. *Mol. Biol. Evol.* **17,** 519–529 (2000).
52. Heimberg, A. M., Cowper-Sal-lari, R., Semon, M., Donoghue, P. C. & Peterson, K. J. microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. *Proc. Natl Acad. Sci. USA* **107,** 19379–19383 (2010).
53. Ziermann, J. M., Miyashita, T. & Diogo, R. Cephalic muscles of Cyclostomes (hagfishes and lampreys) and Chondrichthyes (sharks, rays and holocephalans): comparative anatomy and early evolution of the vertebrate head. *Zool. J. Linn. Soc.* **172,** 771–802 (2014).
54. Adachi, N. & Kuratani, S. Development of head and trunk mesoderm in the dogfish, *Scyliorhinus torazame*: I. Embryology and morphology of the head cavities and related structures. *Evol. Dev.* **14,** 234–256 (2012).
55. Adachi, N., Takechi, M., Hirai, T. & Kuratani, S. Development of the head and trunk mesoderm in the dogfish, *Scyliorhinus torazame*: II. Comparison of gene expression between the head mesoderm and somites with reference to the origin of the vertebrate head. *Evol. Dev.* **14,** 257–276 (2012).
56. Kuratani, S., Adachi, N., Wada, N., Oisi, Y. & Sugahara, F. Developmental and evolutionary significance of the mandibular arch and prechordal/premandibular cranium in vertebrates: revising the heterotopy scenario of gnathostome jaw evolution. *J. Anat.* **222,** 41–55 (2013).
57. Kusakabe, R., Kuraku, S. & Kuratani, S. Expression and interaction of muscle-related genes in the lamprey imply the evolutionary scenario for vertebrate skeletal muscle, in association with the acquisition of the neck and fins. *Dev. Biol.* **350,** 217–227 (2011).
58. Kokubo, N. *et al*. Mechanisms of heart development in the Japanese lamprey, *Lethenteron japonicum*. *Evol. Dev.* **12,** 34–44 (2010).
59. Onimaru, K., Shoguchi, E., Kuratani, S. & Tanaka, M. Development and evolution of the lateral plate mesoderm: comparative analysis of amphioxus and lamprey with implications for the acquisition of paired fins. *Dev. Biol.* **359,** 124–136 (2011).
60. Sauka-Spengler, T., Le Mentec, C., Lepage, M. & Mazan, S. Embryonic expression of *Tbx1*, a DiGeorge syndrome candidate gene, in the lamprey *Lampetra fluviatilis*. *Gene Expr. Patterns* **2,** 99–103 (2002).
61. Tiecke, E. *et al*. Identification and developmental expression of two *Tbx1/10*-related genes in the agnathan *Lethenteron japonicum*. *Dev. Genes Evol.* **217,** 691–697 (2007).
62. Simões-Costa, M. S. *et al*. The evolutionary origin of cardiac chambers. *Dev. Biol.* **277,** 1–15 (2005).
63. Moorman, A. F. & Christoffels, V. M. Cardiac chamber formation: development, genes, and evolution. *Physiol. Rev.* **83,** 1223–1267 (2003).
64. Ziermann, J. M. & Diogo, R. Cranial muscle development in the model organism *Ambystoma mexicanum*: implications for tetrapod and vertebrate comparative and evolutionary morphology and notes on ontogeny and phylogeny. *Anat. Rec. (Hoboken)* **296,** 1031–1048 (2013).
65. Matsuoka, T. *et al*. Neural crest origins of the neck and shoulder. *Nature* **436,** 347–355 (2005).
66. Ziermann, J. M. & Diogo, R. Cranial muscle development in frogs with different developmental modes: direct development versus biphasic development. *J. Morphol.* **275,** 398–413 (2014).
67. Shearman, R. M. & Burke, A. C. The lateral somitic frontier in ontogeny and phylogeny. *J. Exp. Zool. B Mol. Dev. Evol.* **312,** 603–612 (2009).
68. Minchin, J. E. *et al.* Oesophageal and sternohyal muscle fibres are novel Pax3-dependent migratory somite derivatives essential for ingestion. *Development* **140,** 2972–2984 (2013).
69. Abdala, V. & Diogo, R. Comparative anatomy, homologies and evolution of the pectoral and forelimb musculature of tetrapods with special attention to extant limbed amphibians and reptiles. *J. Anat.* **217,** 536–573 (2010).
70. Edgeworth, F. H. *The Cranial Muscles of Vertebrates* (The University Press, Cambridge 1935).
This 80-year-old publication continues to be the most complete compendium on the anatomical development of the head muscles of vertebrates.

71. Piotrowski, T. & Nusslein-Volhard, C. The endoderm plays an important role in patterning the segmented pharyngeal region in zebrafish (*Danio rerio*). *Dev. Biol.* **225,** 339–356 (2000).
72. Noden, D. M. & Schneider, R. A. Neural crest cells and the community of plan for craniofacial development: historical debates and current perspectives. *Adv. Exp. Med. Biol.* **589,** 1–23 (2006).
73. Theis, S. *et al.* The occipital lateral plate mesoderm is a novel source for vertebrate neck musculature. *Development* **137,** 2961–2971 (2010).
74. Gegenbaur, C. *Elements of Comparative Anatomy* (Macmillan, 1878).
75. Gillis, J. A., Dahn, R. D. & Shubin, N. H. Shared developmental mechanisms pattern the vertebrate gill arch and paired fin skeletons. *Proc. Natl Acad. Sci. USA* **106,** 5720–5724 (2009).
76. Putnam, N. H. *et al.* The amphioxus genome and the evolution of the chordate karyotype. *Nature* **453,** 1064–1071 (2008).
77. Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439,** 965–968 (2006).
78. Butler, A. B. The serial transformation hypothesis of vertebrate origins: comment on "The new head hypothesis revisited". *J. Exp. Zool. B Mol. Dev. Evol.* **306,** 419–424 (2006).
79. Gans, C. Stages in the origin of vertebrates: analysis by means of scenarios. *Biol. Rev. Camb. Philos. Soc.* **64,** 221–268 (1989).
80. Mazet, F. *et al.* Molecular evidence from *Ciona intestinalis* for the evolutionary origin of vertebrate sensory placodes. *Dev. Biol.* **282,** 494–508 (2005).
81. Mazet, F. & Shimeld, S. M. Molecular evidence from ascidians for the evolutionary origin of vertebrate cranial sensory placodes. *J. Exp. Zool. B Mol. Dev. Evol.* **304,** 340–346 (2005).
82. Wagner, E. & Levine, M. FGF signaling establishes the anterior border of the *Ciona* neural tube. *Development* **139,** 2351–2359 (2012).
83. Christiaen, L., Bourrat, F. & Joly, J. S. A modular *cis*-regulatory system controls isoform-specific pitx expression in ascidian stomodaeum. *Dev. Biol.* **277,** 557–566 (2005).
84. Christiaen, L. *et al. Pitx* genes in Tunicates provide new molecular insight into the evolutionary origin of pituitary. *Gene* **287,** 107–113 (2002).
85. Abitua, P. B., Wagner, E., Navarrete, I. A. & Levine, M. Identification of a rudimentary neural crest in a non-vertebrate chordate. *Nature* **492,** 104–107 (2012).
86. Satou, Y., Imai, K. S. & Satoh, N. The ascidian *Mesp* gene specifies heart precursor cells. *Development* **131,** 2533–2541 (2004).
87. Davidson, B., Shi, W. & Levine, M. Uncoupling heart cell specification and migration in the simple chordate *Ciona intestinalis*. *Development* **132,** 4811–4818 (2005).
88. Christiaen, L. *et al.* The transcription/migration interface in heart precursors of *Ciona intestinalis*. *Science* **320,** 1349–1352 (2008).
89. Davidson, B., Shi, W., Beh, J., Christiaen, L. & Levine, M. FGF signaling delineates the cardiac progenitor field in the simple chordate, *Ciona intestinalis*. *Genes Dev.* **20,** 2728–2738 (2006).
90. Beh, J., Shi, W., Levine, M., Davidson, B. & Christiaen, L. FoxF is essential for FGF-induced migration of heart progenitor cells in the ascidian *Ciona intestinalis*. *Development* **134,** 3297–3305 (2007).
91. Christiaen, L., Stolfi, A. & Levine, M. BMP signaling coordinates gene expression and cell migration during precardiac mesoderm development. *Dev. Biol.* **340,** 179–187 (2010).
92. Ragkousi, K., Beh, J., Sweeney, S., Starobinska, E. & Davidson, B. A single GATA factor plays discrete, lineage specific roles in ascidian heart development. *Dev. Biol.* **352,** 154–163 (2011).
93. Stolfi, A. *et al.* Early chordate origins of the vertebrate second heart field. *Science* **329,** 565–568 (2010).
This article reports the discovery of the CPF in *C. intestinalis* using dynamic imaging and genetics, revealing striking genetic similarities with vertebrate pharyngeal mesoderm giving rise to head muscles and SHF-derived parts of the heart.
94. Tolkin, T. & Christiaen, L. Development and evolution of the ascidian cardiogenic mesoderm. *Curr. Top. Dev. Biol.* **100,** 107–142 (2012).
95. Wang, W., Razy-Krajka, F., Siu, E., Ketcham, A. & Christiaen, L. NK4 antagonizes Tbx1/10 to promote cardiac versus pharyngeal muscle fate in the ascidian second heart field. *PLoS Biol.* **11,** e1001725 (2013).
This paper identified an ontogenetic motif regulating cardiac and pharyngeal skeletal muscle development in *C. intestinalis* through asymmetric cell division events and anatagonistic interactions between conserved master regulators of cardiopharyngeal fate.
96. Razy-Krajka, F. *et al.* Collier/OLF/EBF-dependent transcriptional dynamics control pharyngeal muscle specification from primed cardiopharyngeal progenitors. *Dev. Cell* **29,** 263–276 (2014).
This paper demonstrated that the multipotent cardiopharyngeal progenitors of *C. intestinalis* are multilineage primed and activate both early heart and pharyngeal muscle regulators that segregate to their corresponding precursors following asymmetric cell divisions.
97. Harafuji, N., Keys, D. N. & Levine, M. Genome-wide identification of tissue-specific enhancers in the *Ciona* tadpole. *Proc. Natl Acad. Sci. USA* **99,** 6802–6805 (2002).
98. Heude, E. *et al.* Jaw muscularization requires Dlx expression by cranial neural crest cells. *Proc. Natl Acad. Sci. USA* **107,** 11441–11446 (2010).
99. Yasui, K., Kaji, T., Morov, A. R. & Yonemura, S. Development of oral and branchial muscles in lancelet larvae of *Branchiostoma japonicum*. *J. Morphol.* **275,** 465–477 (2014).
100. Goldschmidt, R. Amphioxides. *Wiss Ergeb Dtsch Tiefsee-Expedition* [in German] **12,** 1–92 (1905).
101. Holland, N. D., Venkatesh, T. V., Holland, L. Z., Jacobs, D. K. & Bodmer, R. *AmphiNk2-tin*, an amphioxus homeobox gene expressed in myocardial progenitors: insights into evolution of the vertebrate heart. *Dev. Biol.* **255,** 128–137 (2003).
102. Mahadevan, N. R., Horton, A. C. & Gibson-Brown, J. J. Developmental expression of the amphioxus *Tbx1/10* gene illuminates the evolution of vertebrate branchial arches and sclerotome. *Dev. Genes Evol.* **214,** 559–566 (2004).
103. Jackman, W. R., Langeland, J. A. & Kimmel, C. B. *islet* reveals segmentation in the Amphioxus hindbrain homolog. *Dev. Biol.* **220,** 16–26 (2000).
104. Belgacem, M. R., Escande, M. L., Escriva, H. & Bertrand, S. Amphioxus Tbx6/16 and Tbx20 embryonic expression patterns reveal ancestral functions in chordates. *Gene Expr. Patterns* **11,** 239–243 (2011).
105. Willey, A. *Amphioxus and the Ancestery of the Vertebrates* (Macmillan, 1894).
106. Schubert, M., Meulemans, D., Bronner-Fraser, M., Holland, L. Z. & Holland, N. D. Differential mesodermal expression of two amphioxus MyoD family members (AmphiMRF1 and AmphiMRF2). *Gene Expr. Patterns* **3,** 199–202 (2003).
107. Mazet, F., Masood, S., Luke, G. N., Holland, N. D. & Shimeld, S. M. Expression of *AmphiCoe*, an amphioxus COE/EBF gene, in the developing central nervous system and epidermal sensory neurons. *Genesis* **38,** 58–65 (2004).
108. Holland, L. Z., Schubert, M., Kozmik, Z. & Holland, N. D. *AmphiPax3/7*, an amphioxus paired box gene: insights into chordate myogenesis, neurogenesis, and the possible evolutionary precursor of definitive vertebrate neural crest. *Evol. Dev.* **1,** 153–165 (1999).
109. Hirano, T. & Nishida, H. Developmental fates of larval tissues after metamorphosis in ascidian *Halocynthia roretzi*. I. Origin of mesodermal tissues of the juvenile. *Dev. Biol.* **192,** 199–210 (1997).
110. Tokuoka, M., Satoh, N. & Satou, Y. A bHLH transcription factor gene, *Twist-like1*, is essential for the formation of mesodermal tissues of *Ciona* juveniles. *Dev. Biol.* **288,** 387–396 (2005).
111. Kuratani, S. Evolution of the vertebrate jaw from developmental perspectives. *Evol. Dev.* **14,** 76–92 (2012).
112. Mallatt, J. The origin of the vertebrate jaw: neoclassical ideas versus newer, development-based ideas. *Zoolog. Sci.* **25,** 990–998 (2008).
113. Valentine, J. W. *On the Origin of Phyla* (Univ. Chicago Press, 2004).
114. Gillis, J. A., Fritzenwanker, J. H. & Lowe, C. J. A stem-deuterostome origin of the vertebrate pharyngeal transcriptional network. *Proc R. Soc. B* **279,** 237–246 (2012).
115. Haun, C., Alexander, J., Stainier, D.Y. & Okkema, P. G. Rescue of *Caenorhabditis elegans* pharyngeal development by a vertebrate heart specification gene. *Proc. Natl Acad. Sci. USA.* **95,** 5072–5075 (1998).
116. Boukhatmi, H. *et al.* An Org-1-Tup transcriptional cascade reveals different types of alary muscles connecting internal organs in *Drosophila*. *Development* **141,** 3761–3771 (2014).
117. Crozatier, M. & Vincent, A. Requirement for the *Drosophila* COE transcription factor Collier in formation of an embryonic muscle: transcriptional response to notch signalling. *Development* **126,** 1495–1504 (1999).
118. Enriquez, J., de Taffin, M., Crozatier, M., Vincent, A. & Dubois, L. Combinatorial coding of *Drosophila* muscle shape by Collier and Nautilus. *Dev. Biol.* **363,** 27–39 (2012).
119. Mann, T., Bodmer, R. & Pandur, P. The *Drosophila* homolog of vertebrate *Islet1* is a key component in early cardiogenesis. *Development* **136,** 317–326 (2009).
120. Schaub, C. & Frasch, M. Org-1 is required for the diversification of circular visceral muscle founder cells and normal midgut morphogenesis. *Dev. Biol.* **376,** 245–259 (2013).
121. Schaub, C., Nagaso, H., Jin, H. & Frasch, M. Org-1, the *Drosophila* ortholog of Tbx1, is a direct activator of known identity genes during muscle specification. *Development* **139,** 1001–1012 (2012).
122. Lescroart, F. & Meilhac, S. M. Cell lineages, growth and repair of the mouse heart. *Results Probl. Cell Differ.* **55,** 263–289 (2012).
123. Lescroart, F., Mohun, T., Meilhac, S. M., Bennett, M. & Buckingham, M. Lineage tree for the venous pole of the heart: clonal analysis clarifies controversial genealogy based on genetic tracing. *Circ. Res.* **111,** 1313–1322 (2012).
124. Lacalli, T. C. & Holland, L. Z. The developing dorsal ganglion of the salp *Thalia democratica*, and the nature of the ancestral chordate brain. *Phil. Trans. R. Soc. Lond. B* **353,** 1943–1967 (1998).
125. Gee, H. in *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development* (ed. Ahlberg, P. E.) 1–14 (Taylor & Francis, 2001).

Acknowledgements We thank T. Miyashita and F. Razy-Krajka for their detailed reviews of the manuscript. We are thankful to the Dean of Howard University (HU) College of Medicine, M. Johnson, and the Chair of HU Department of Anatomy, D. Orlic, for helping to organize, financially and logistically, the First Evo-Devo Meeting On Heart and Head Muscles at HU (May, 2014) that led to the publication of this Review. We also thank the other participants at the workshop: A. Kahana, P. Okkema, A. Vincent, T. Hirasawa, S. Tajbakhsh, S. Dietrich and R. Knight. L.C. is supported by National Institutes of Health (NIH)/National Institute of General Medical Sciences (NIGMS) grant R01GM096032 and NIH/National Heart, Lung and Blood Instiute (NHLBI) grant R01HL108643, E.T. by the European Research Council and Israel Science Foundation, R.D. and J.Z. by HU College of Medicine, R.G.K. by Inserm, the Agence Nationale pour la Recherche, Association Française contre les Myopathies and Fondation pour la Recherche Médicale, and M.L. by NIH grant NS076542.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this paper at go.nature.com/wy4cga. Correspondence should be addressed to R.D. (rui.diogo@howard.edu), R.G.K. (Robert.Kelly@univ-amu.fr), L.C. (lc121@nyu.edu) or E.T. (eldad.tzahor@weizmann.ac.il).

REVIEW

doi:10.1038/nature14436

Evolution of vertebrates as viewed from the crest

Stephen A. Green[1], Marcos Simoes-Costa[1] & Marianne E. Bronner[1]

The origin of vertebrates was accompanied by the advent of a novel cell type: the neural crest. Emerging from the central nervous system, these cells migrate to diverse locations and differentiate into numerous derivatives. By coupling morphological and gene regulatory information from vertebrates and other chordates, we describe how addition of the neural-crest-specification program may have enabled cells at the neural plate border to acquire multipotency and migratory ability. Analysis of the topology of the neural crest gene regulatory network can serve as a useful template for understanding vertebrate evolution, including elaboration of neural crest derivatives.

The vertebrate body plan emerged in concert with extensive changes to anterior chordate morphology, including assembly of a craniofacial skeleton, expansion of the anterior neuroepithelium into a brain, reorganization of the pharynx and appearance of novel sensory systems[1–3]. The genesis of this vertebrate 'new head'[1] has been fundamentally linked to the emergence of two cell types, neural crest cells and ectodermal placodal cells. The neural crest is a transient vertebrate cell type, characterized by its site of origin within the central nervous system (CNS), multipotency, and its ability to migrate and differentiate into numerous derivatives, as diverse as cartilage, bone, melanocytes, peripheral neurons and glia[4]. Together with ectodermal placodes that give rise to the sense organs of the head (see refs 5, 6 for discussion of placode evolution), neural crest cells have contributed to the remarkable array of novel anatomies that make vertebrates unique.

Neural crest cells are unlike any other cell type, and the advent of this progenitor cell population affected chordate evolution in an unprecedented manner. Although cells with subsets of neural crest characteristics are present in invertebrate chordates, only vertebrates have a bona fide neural crest that gives rise to structural elements of the head, glia, pigment cells and neurons. Imbued with broad developmental potential and extensive migratory ability, neural crest cells have gained developmental roles at nearly all axial levels and extensively interact with many other tissues. For these reasons, the neural crest is often referred to as the fourth germ layer[7], associated with the emergence and elaboration of the vertebrate body plan[1,8,9].

In this Review, we examine the morphological and genetic features that distinguish vertebrates from other chordates, focusing on cells and tissues derived from the neural crest. We place special emphasis on contributions that resulted in the assembly of the vertebrate head, which has played a crucial part in establishment and diversification of vertebrates. We discuss the gene regulatory network (GRN) underlying the formation of the early neural crest cells that are common to all vertebrates. We then use this network, together with morphological criteria, to discuss how neural crest cells may have emerged from the putative homologues that are present in invertebrate chordates, highlighting how addition of the neural-crest-specification program may have enabled cells at the CNS border to acquire multipotency and migratory ability. In this context, we examine how studies of neural crest GRNs may clarify patterns of morphological evolution within vertebrates, including expansion of neural crest derivatives during diversification of vertebrate taxa. Taken together, the data paint a picture of the neural crest as a malleable population that has continued to imbue the vertebrate body with novel features.

Neural-crest-related innovations in early vertebrates

Emergence of the vertebrate lineage was accompanied by acquisition of the neural crest and its novel derivatives. All vertebrates have neural crest cells that arise from the dorsal portion of the CNS, exhibit multipotency by contributing to diverse derivatives, undergo an epithelial-to-mesenchymal transition (EMT), and have extensive migratory ability. 'Premigratory' neural crest cells initially reside in or adjacent to the dorsal neural tube, the newly formed CNS, of all vertebrates[10]. These cells undergo EMT to exit the CNS and migrate to numerous sites throughout the body, where they eventually contribute to their characteristic derivatives[4] (Fig. 1a). Cell-lineage analyses have shown that many individual neural crest precursors can contribute to multiple cell types *in vivo*[11–13] and *in vitro*[14,15], and are thus 'multipotent' stem or progenitor cells.

Comparisons between the two major groups of living vertebrates, the jawed vertebrates (gnathostomes) and their sister group the cyclostomes (agnathans)[16], identify many shared, derived traits likely to have been present in the neural crest of early vertebrates[17–20]. These include pigment cells, cellular pharyngeal cartilage and specialized pharyngeal musculature, an enteric nervous system, chromaffin cells, and perhaps cardiac valves[17,21]. Recent work has identified a new neural crest derivative, pillar cells[22], that support vertebrate gill epithelia (Box 1). Because neural crest cells interact with many other tissues, they have a broad impact by modifying neuroepithelial patterning, craniofacial patterning, and cranial musculoskeletal development (Box 2).

Many early vertebrate innovations are unique to jawed vertebrates and absent in cyclostomes. Some of these traits are likely to have arisen in stem gnathostomes, the early fishes that are ancestral to the jawed vertebrates. One of these innovations is the appearance of jaws, through modification of anterior pharyngeal arches. Other major gnathostome innovations include odontoblasts that produce dentine (Box 1), paravertebral sympathetic chain ganglia[23] (Box 3) and exoskeletal armour. Although exoskeletal armour might have arisen from neural crest at cranial levels, it is likely that trunk armour instead arose from mesoderm (Box 4).

One central question in the early evolution of neural crest is the extent to which neural crest cell types are evolutionary novelties, rather than cell types (and regulatory programs) co-opted from other tissues. There are clearly some novel neural-crest-derived cell types, including

[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California 91125, USA.

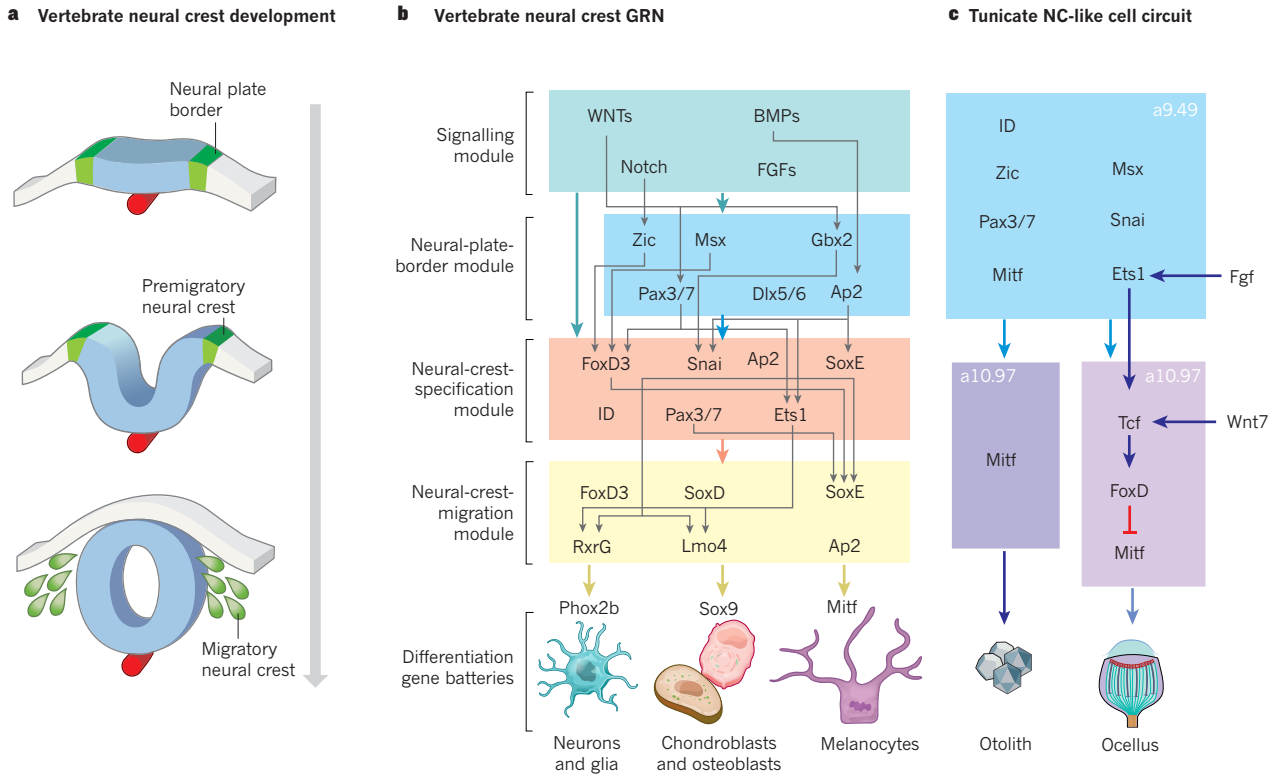


Figure 1 | Gene regulatory interactions controlling vertebrate neural crest formation and the tunicate a9.49 cell lineage. **a**, Different stages in neural crest formation. Neural crest cells are defined by their origin at the neural plate border, epithelial to mesenchymal transition, migratory capacity and multipotency. **b**, A neural crest gene regulatory network (GRN) endows this cell population with its unique features. This GRN is composed of different modules arranged hierarchically, which control each step of neural crest development[38]. The neural-crest-specification module seems to be missing from the neural plate border of invertebrate chordates. **c**, Regulatory circuit of a tunicate neural-crest (NC)-like pigmented cell precursor. Adapted from refs 38, 39 and based on the results from ref. 49.

pillar cells and odontoblasts, but many neural crest cell types are similar to cells in related chordates[24,25]. These cell types might either be homologous, representing a cell lineage that was co-opted and incorporated into the neural crest, or they might have arisen by convergent evolution. One example of co-option is the origin of pharyngeal cellular cartilage, probably accomplished by reuse of a program governing cellular cartilage formation in the oral region of invertebrate chordates[26]. Assessment of co-option or novelty can be aided by evaluation of GRNs that govern their formation.

A neural crest GRN is conserved across vertebrates

From a gene regulatory perspective, the body plan of all metazoans is encoded in the genome. During embryonic development, this code emerges as a complex GRN formed by transcription factors and *cis*-regulatory elements that co-operate with non-coding RNAs and epigenetic factors to pattern the body and drive development of individual elements and cell types[27]. According to this framework, the body-plan modifications observed during evolution are a direct consequence of changes in the developmental regulatory program[28].

Neural crest cells are characterized by site of origin, migratory behavior and multipotency. Importantly, they also share a molecular signature, expressing a suite of transcription factors, including *tfAP2* (ref. 29), *Snai1/2* (ref. 30), *FoxD3* (refs 31–33) and *SoxE* (refs 34, 35) genes. In particular, *FoxD3* and *SoxE* are characteristic of premigratory and early migratory neural crest cells and *SoxE* genes are crucial upstream regulators of all neural crest lineages. These transcription factors are part of the regulatory machinery that controls transcription of numerous effector genes, which together endow the neural crest with its unique properties. Interactions between transcription factors and their targets generate a GRN that controls neural crest formation, from induction at the neural plate border to differentiation into distinct cell types[36–39] (Fig. 1b).

The architecture of the neural crest GRN is thought to underlie the features observed in this cell population, such as multipotency and migratory capability. Functional experiments suggest that the neural crest GRN is comprised of distinct hierarchical levels[36,38]. First, signalling events (GRN signalling module) initiate the specification process, by inducing co-expression of transcription factors that comprise the 'neural-plate-border module'. This in turn leads to specification of bona fide neural crest cells (neural-crest-specification module), their migration from the CNS to diverse sites (neural-crest-migration module), and finally to diversification into different derivatives through the deployment of distinct differentiation gene batteries[36–39] (Fig. 1b). Each level of the neural crest GRN corresponds to a regulatory state that not only defines cell identity and behaviour at a given time point, but also drives transition to the next module of the network[40]. From an evolutionary perspective, assessing conservation of different levels of the neural crest GRN helps to identify the origin of each subcircuit and reconstruct the evolutionary history of neural crest cells[27,28]. As a result, the neural crest GRN provides a useful platform for understanding the molecular underpinnings of vertebrate evolution and how these cells may have participated in modifying vertebrate embryonic development. Neural-crest-GRN studies have indeed provided important clues regarding the establishment of the vertebrate lineage and its diversification[40–42].

Extensive work in amniotes, frogs, teleosts and cyclostomes has revealed remarkable similarities in the overall structure of the neural crest GRN, demonstrating that it is virtually the same from amniotes to cyclostomes (Fig. 1b)[8,10,19,43]. Some important species-specific differences exist, but they are likely to reflect the continuous restructuring of the GRN in individual clades. Nevertheless, expression patterns and epistatic interactions between FoxD3, SoxE, Snai1/2 and Pax3/7 transcription factors point to a very conserved module of neural crest specification[38]. The overall conservation of the neural crest GRN correlates with conservation

BOX 1

Neural crest derivatives and the vertebrate pharynx

Changes in pharyngeal patterning are central to the evolution and diversification of vertebrate groups[1,98]. Vertebrate pharyngeal arches have a similar general structure, characterized as a bilaterally symmetric series of endodermal evaginations that, with ectoderm, enclose a region of neural crest cells surrounding paraxial mesoderm[99,100]. Neural crest cells and paraxial mesoderm give rise to pharyngeal skeletal elements and musculature, respectively.

Some aspects of vertebrate pharyngeal patterning are integrated within or modified from features common to many deuterostomes. Pharyngeal segmentation is a trait of ancestral deuterostomes[101], and unambiguous pharyngeal arch homologues with similar genetic controls are present in hemichordates, cephalochordates and adult urochordates[99,101], despite being secondarily lost in echinoderms[99,102]. Pharyngeal mesoderm also has a broad phylogenetic distribution, being present throughout chordates[103,104]. Neural-crest-derived cellular cartilage of vertebrates, rather than being a novelty of vertebrates[21], instead seems to have been co-opted from cellular cartilage homologous to that present within the oral cirri of cephalochordates[26].

Although some vertebrate pharyngeal patterning stems from ancestral conditions, many novel elements arise from vertebrate neural crest cells. Modification of early neural crest development was important for generating the diversity of pharyngeal structures observed throughout vertebrates. For example, in vertebrate gills, epithelial surfaces are supported by novel neural-crest-derived cells, pillar cells, which are ancestrally shared throughout vertebrates[22]. In addition, in the transition from agnathans to gnathostomes, modifications to the anterior-most pharyngeal arch cartilages and neural-crest-modified musculature resulted in the formation of the jaws, as well as the formation of neck muscles[18,105–107].

Another vertebrate novelty associated with the pharynx and its integuments are odontodes: dental elements composed of mineral material and associated cells. In living jawed vertebrates, their formation is mediated by conserved gene regulatory subcircuits, identified by coexpression of transcription factors, including runx2 and eda/edar, among others[108], and require the inductive influence of neural-crest-derived mesenchyme. Fossil evidence suggests that odontodes emerged during the evolution of stem gnathostomes, in external dermal armour[108–110], consistent with the 'outside–in' model, which suggests that odontodes emerged first as structural elements associated with external integument, and were later incorporated into the oral cavity and pharynx. Mineralized dental elements found in conodont fossils are considered non-homologous to gnathostome teeth[109]. Both groups of living cyclostomes, lampreys and hagfish, have keratinized dental elements, but these are morphologically distinct from gnathostome teeth and are probably not homologous. Continued analysis of cyclostome dental elements might clarify whether neural crest cells played a part in their ontogeny.

of morphology, migratory behaviour and differentiation into multiple derivatives, establishing the neural crest as an ancient vertebrate cell type. Superimposed on the conserved basic structure of the neural crest GRN is adaptability and flexibility. During the course of evolution, differentiation modules that encode for novel derivatives, such as jaws and sympathetic ganglia, have been added to the neural crest repertoire and thus must have been added as 'plug-ins' to the GRN.

Although the core elements are highly conserved, adaptations, additions and potentially losses have occurred between species. Indeed, it is clear that the specification module of the neural crest GRN is strongly conserved within vertebrates, but there are important gene regulatory differences between jawless and jawed vertebrates that might provide interesting hints regarding the molecular roots of vertebrate morphological diversification. Extensive analysis of the lamprey neural crest GRN has revealed the notable absence of transcription factors *Ets-1* and *Twist* in the premigratory neural crest[10]. This is intriguing since Ets-1 has been shown to be essential for cranial neural crest specification in gnathostomes[34]. Instead, in the lamprey, it is expressed much later in the neural-crest-derived portion of the branchial arches and dorsal root ganglia. One possibility is that Ets-1 was added to the gnathostome neural crest specification, representing an example of a transcription factor that was co-opted from a distal level of the network to a more proximal level. However, it is also possible that it may have been selectively lost in the lamprey neural crest. Examining expression of Ets-1 in other cyclostomes and functional experiments in lampreys may help to clarify this point. Other GRN components that have crucial functions in teleosts and amphibians may have been lost or replaced in amniotes. For example, although Snai1/2 and Twist seem to be crucial for neural crest formation in frogs[44,45], they are dispensable in mice[46], perhaps due to redundant functions with other EMT factors such as Sip1 (ref. 47).

Taken together, these studies reveal that the topology of the neural crest GRN, with cells progressing through successive regulatory states from induction to differentiation, forms a useful template for understanding vertebrate evolution[36]. This GRN can also be useful for assessing the likelihood that similar cell types in other animals might be homologous to the neural crest.

Do invertebrate chordates have neural crest cells?

Deciphering how the neural crest arose as a cell type is crucial for furthering our understanding of vertebrate evolution. Tackling this problem requires deeper knowledge of deuterostome embryonic development in multiple species, with particular attention to neural-crest-like cell types in other chordates. Recent studies have described intriguing embryonic cell populations in ascidians that have some, but not all, neural crest characteristics. For example, the trunk lateral cells in the colonial tunicate *Ecteinascidia turbinata* are derived from the A7.6 lineage, which originates in the vicinity of the neural tube, undergoes migration and gives rise to pigmented cell types[48]. Similarly, in *Ciona intestinalis*, results show that the a9.49 cell lineage originates from the neural plate border and gives rise to the pigmented sensory cells of the otolith and the ocellus[49]. These cells normally translocate only a few cell diameters, whereas misexpression of Twist in this lineage results in acquisition of mesenchymal morphology and long-range migration[49]. In cephalochordates, there have been many proposed homologues of neural crest (see ref. 50 for a discussion), including a bipotential neuroepithelial precursor to pigment cells of the ocellus[50]. Further assessment of this homology will require additional analyses of amphioxus ocellus development. Cephalochordates also have an ependymal cell in the neural tube that expresses *Snail*, a homologue of *Snai1* and a neural-crest-specifier gene in vertebrates, but this cell seems to be non-migratory[51,52].

The neural crest GRN is particularly useful for understanding assessment of GRN conservation outside of vertebrates. The available molecular data obtained from embryonic cell types in tunicates and cephalochordates suggest that gene regulatory interactions that specify the neural plate border (neural-plate-border module) are deeply conserved throughout chordates[24,51] (Fig. 1c), and data from annelids suggest that this genetic program might be shared with protostomes, originating in stem bilaterians[53,54]. Similarly, the terminal differentiation programs (differentiation gene batteries) that drive the neural crest to assume definitive fates are

conserved, as exemplified by control of pigment-cell differentiation. This is expected because most of the differentiation batteries are thought to be ancient subcircuits that were co-opted by different cell types[27]. Although they are integral parts of the neural crest GRN, these neural-plate-border and differentiation subcircuits do not fully define neural crest identity in vertebrates. Proximally in the program, the neural plate border contains other cell types (neural tube and placode) in addition to neural crest, and is important for the delimitation of the neural plate. Distally, other deuterostomes have some differentiated cell types that in vertebrates can arise from neural crest: melanocytes, ectomesenchyme, autonomic neurons and glia. It has been proposed that during early vertebrate evolution, the neural-crest-specification module may have been assembled within the neural-plate-border cell lineage, interposed between the neural plate border and the distal differentiation modules of the network, to endow these cells with a full 'neural crest' phenotype.

Importantly, neural crest identity in all vertebrates is intrinsically linked to the neural-crest-specification kernel of the GRN, which endows these cells with its defining features such as multipotency, the ability to undergo EMT and migratory capacity[40]. Important genes in the specification subcircuit include *SoxE*, *FoxD* and *Snai1/2*, homologues of which are present in the genomes of invertebrate chordates[51,55]. For example, the amphioxus genome has all the transcription factors identified in the neural-crest-specifier module of the vertebrate neural crest GRN. However, only *AmphiSnail* is expressed in the putative neural crest domain[56]. Therefore, a key question is whether the neural-crest-like cells from tunicates possess this particular subcircuit. Molecular analyses suggest that tunicates and amphioxus have the neural-plate-border subcircuit[24], and thus invertebrate neural-crest-like cells may be homologous to neural-plate-border cells of vertebrates. However, although some neural-plate-specifier genes are expressed in these cells (for example, *FoxD*[49]) other crucial transcription-factor genes, notably *SoxE* genes, seem to be absent. In ascidians, it is not yet clear whether epistatic interactions between the transcription factors expressed in putative neural crest cells are similar to those observed in the vertebrate neural crest GRN (Fig. 1c). This, together with the fact that cells of the a9.49 lineage have not yet been shown to be multipotent, or to have extensive migratory capabilities, makes it more difficult to determine whether they are true neural crest homologues. Further gene-regulatory studies will be necessary to establish the relationship between these cells and the vertebrate neural crest.

As a cautionary note, there is inherent danger in assigning evolutionary relationships among cell types on the basis of molecular similarity alone, because transcription factors are reused throughout development, and are neither lineage- nor cell-type-specific. For instance, many bona fide neural crest transcription factors are expressed at the neural plate border, in later differentiation programs and in other lineages. Thus, one cannot attribute homology or lineage relationships on the basis of a few molecular markers. A more inclusive argument that includes morphological and behavioural information, expression data and, ideally, *cis*-regulatory studies[57] perhaps provides the most reliable means to establish conservation of developmental mechanisms and ascribe homology between cell populations.

Gene regulatory changes behind neural crest emergence

Radical changes of body plan, such as those that took place in early vertebrate evolution, require substantial rearrangements in the structure of developmental GRNs[27]. The emergence of the neural crest was dependent on the assembly of a specification subcircuit that allowed this cell population not only to exhibit its stereotypical behaviour, but also to drive multiple differentiation programs, resulting in its multipotent state. Understanding how a novel, complex specification subcircuit emerged during chordate evolution is a daunting task. However, observation of the neural crest GRN can provide important clues about vertebrate evolution and suggest likely scenarios for the creation of a novel cell type.

Given the deep conservation of the neural-plate-border-specification program[24], it seems reasonable to assume that this circuit was crucial for assembly of the vertebrate neural crest GRN. Because all of the neural-crest-specifier genes are present in the genomes of invertebrate chordates[58,59], it is likely that they were added to the GRN by deployment or co-option of transcription factors that were originally part of other developmental GRNs, such as the neural-plate-border subcircuit, mesodermal programs and terminal differentiation modules. According to this view, changes in their *cis*-regulatory apparatus placed the neural-crest-specifier genes downstream of the neural-plate-border program and signalling systems. Such *cis*-regulatory changes might have facilitated redeployment of neural-plate-border (*Pax3/7* and *TFAP2*) and stem-cell genes (*FoxD3*) in the specification module. For example, an amphioxus *FoxD* enhancer that recapitulates endogenous amphioxus *FoxD* expression

BOX 2

Role of the neural crest in signalling

Brain and facial patterning. Increased complexity in vertebrate neuroanatomy might partly stem from interactions between neural crest cells and other cell types. An example of the important role of the neural crest in expansion of the head comes from recent experiments in amniotes[111]. Surgical removal of the neural crest at forebrain to rostral hindbrain levels results in the absence of facial and skull cartilages and bones, as well as severe brain defects including anencephaly[112]. These defects can be rescued by grafting small populations of premigratory neural crest from the same axial level, but not from more caudal regions with Hox gene expression. At a molecular level, this results from production of BMP inhibitors, Gremlin and Noggin, by the rostral neural crest that in turn lead to regulation of expression of FGF8 in the anterior neural ridge (ANR). Consistent with this, implantation of FGF8 beads after neural crest ablation rescues this phenotype to restore subsequent downstream signalling events and proper head development[100,113]. FGF signalling associated with an ANR-like signalling centre is potentially present throughout deuterostomes[114,115], suggesting that neural crest cells have adopted or co-opted roles in the regulation of neural or craniofacial patterning, at least in amniotes. Examination of additional vertebrate groups might clarify when this might have arisen.

Cranial muscles and the neural crest. The vertebrate head includes muscles that control the movement of the eyes (extraocular muscles), face, jaws, throat, larynx and tongue, collectively called branchiomeric muscles[116]. Derived from unsegmented paraxial mesoderm anterior to the otic vesicle, they form under the control of a Pitx2c and Tcf21/MyoR regulatory subcircuit that seems to be conserved at least throughout the bony fishes[117,118] (Fig. 2). The neural crest is crucial for multiple stages of cranial mesoderm development, including defining the location, orientation, patterning and differentiation state of muscle precursor cells[57,106,107,116]. Mesoderm cells follow migrating neural crest cells into the pharyngeal arches[86,116]. Branchiomeric muscles initially remain in a precursor state, repressed by signals emanating from the nearby neural tube and ectoderm. Neural crest cells secrete signals that derepress myogenesis, allowing the formation of cranial myofibres[119]. These distinct myogenic regulatory sub-networks are thought to have arisen in early vertebrates concurrent with other cephalic modifications[117,119], but have also been compared with muscle precursors in the amphioxus atrium[104] and potentially with visceral musculature of protostomes[120]. Vertebrate cranial muscle patterning, differentiation and organization might require regulatory control that arose from novel interactions with the neural crest (Fig. 2).

BOX 3

Peripheral nervous system

A peripheral nervous system, including the sympathetic chain ganglia, is a common feature of all jawed vertebrates. Sympathetic ganglion cells are responsible for regulating homeostatic functions of peripheral organs. They arise from neural crest cells that migrate ventrally from the trunk neural tube to positions adjacent to the dorsal aorta, and form under the control of a gene regulatory circuit including *Phox2*, *Hand2* and *Ascl1*. These genes collaborate to promote the construction of a sympathetic neural phenotype, including production of noradrenaline. In bony fishes and tetrapods, sympathetic ganglia are connected along the anteroposterior axis through chains, but in extant chondrichyans (sharks, rays and skates) ganglia are largely separate. Cyclostomes do not seem to have a comparably organized sympathetic system, but very rare ganglion-like cells of unknown function have been identified[121]. In general, autonomic function in cyclostomes seems to be controlled directly by spinal neurons of the central nervous system[121], which is similar to the peripheral organization of amphioxus, and thus is likely to represent a primitive condition for chordates. Taken together, these data suggest that sympathetic ganglia probably evolved in stem gnathostomes, and were further elaborated in stem osteichthyes.

in somites and notochord[60] was able to drive similar expression when electroporated into chick embryos[51]. However, this enhancer failed to drive expression in the neural crest, suggesting that the novel neural crest expression domains rely on distinct gene regulatory processes that are absent in amphioxus[51]. Similarly, co-option of EMT driver genes such as *Snai2* (ref. 30) and *Sip1* (ref. 47) may have allowed the neural crest to leave the neural plate border domain. This was probably accompanied by co-option of mesenchymal gene circuits that allowed these cells to exhibit migratory behaviour.

A key feature of the neural crest is its ability to form numerous derivatives (multipotency). Mechanistically, this implies that neural crest cells are capable of deploying a variety of differentiation gene batteries depending on signalling interactions during migration and once at their final sites. Neural-crest-specifier genes from the SoxE family play a crucial part in activating differentiation programs that lead to multiple derivatives, as diverse as neurons, Schwann cells, pigment cells and cartilage[38]. Thus, a likely scenario was that a variety of differentiation gene batteries were placed downstream of the neural-crest-specification module by gain of function *cis*-regulatory changes, which placed differentiation driver genes (for example, *Mitf*, *Ascl1* or *Phox2b*) under the control of neural-crest-specifier genes. Again, examples of redeployment of such ancient differentiation gene batteries by different cell types have been described in different contexts, and are thought to be a common feature in GRN evolution[27,61]. Indeed, a recent study[26] suggests that *cis*-regulatory changes in ancestral pro-chondrocytic genes allowed for their activation in the neural crest by factors such as SoxE and Tfap2, allowing for the establishment of the vertebrate head skeleton. Thus, it is possible that the emergence of the neural-crest-specifier module served as a platform for the redeployment of multiple, pre-existing genetic subcircuits that endowed the neural crest with its defining features.

Although *cis*-regulatory changes were probably the most important events in the emergence of the neural-crest-specification module, it is also likely that changes in protein sequence had an important role therein. Neural crest cells employ a large repertoire of adhesion molecules, receptors and signalling molecules, and gene diversification and neofunctionalization might have enabled acquisition of the complex cell behaviours exhibited by the neural crest. Furthermore, recent data suggest that neofunctionalization of neural-crest-specifier genes such as *FoxD3* was important for the emergence of this cell type[62], perhaps by mediating new protein–protein interactions and allowing for the assembly of novel, vertebrate-specific transcriptional complexes.

A role for gene duplications in early neural crest evolution

The extensive changes in gene regulation required for the evolution of the neural crest as a cell type might have been facilitated by large-scale genome duplications that took place early in the vertebrate lineage. It has long been suspected that rare, large-scale genomic rearrangements and genome-wide duplications in stem vertebrates had a key role in elaborating the vertebrate body plan[54,63–65] and increasing vertebrate complexity[66,67]. The presence of multiple homologous Hox clusters and conserved syntenic paralogy regions among jawed vertebrate chromosomes are usually taken to support the contention that there were two rounds of genome duplication during early vertebrate evolution[66]. Recent analysis of the genome of the sea lamprey (*Petromyzon marinus*) suggested that ancestors of the lamprey (and hagfish) diverged from vertebrates after these two rounds of duplication[68–70], but this is still controversial, and an alternative model suggests that there was only a single round of duplication in stem vertebrates, followed by lineage-specific segmental duplications in jawed vertebrates and cyclostomes[71]. Regardless of the precise number and timing of genome duplications, vertebrates have certainly undergone additional gene duplications relative to invertebrates, and these increases in gene number may have facilitated the evolution of vertebrate regulatory and anatomic complexity[63], potentially affecting the formation of the many novel cell types in vertebrates.

A full assessment of the extent to which gene and genome duplications have affected early vertebrate evolution remains incomplete, and is somewhat controversial[72]. One way to approach this question is to determine whether the timing of the acquisition of particular traits compares with the inferred timing of gene duplications. Many traits were thought to arise in the vertebrate stem: these include key innovations such as the addition of neural-crest-derived pharyngeal cartilages, modification of cranial muscles, the development of segmented and Hox-patterned hindbrain[57], and perhaps the beginnings of peripheral nervous organization (Fig. 2). These distinct vertebrate characters are rooted in invertebrate chordates, but seem to have been fundamentally transformed by the innovation of neural crest cells and their interactions with other cell types. Thus, the timing of the acquisition of these traits correlates nicely with inferred instances of genome duplication, although one cannot distinguish cause from effect.

Ultimately, the fundamental question is how genomic duplications affected the organization of developmental GRNs. As has been discussed[54], such duplications may cause important shifts in gene regulatory mechanisms during vertebrate evolution. Indeed, it is possible that large-scale genome duplications may have facilitated extensive changes in the *cis*-regulatory apparatus controlling the transcription of neural crest genes[73], leading to their co-option and assembly into the neural-crest-specification module. Such events might have enabled the deployment of genes, such as those that encode SoxE transcription factors, in the neural-crest-specification module. Depending on the species, *Sox8*, *Sox9* and *Sox10* have early and sometimes overlapping functions in neural crest specification, with different paralogues deployed at different times depending on the species. However, expressing at least one of the *SoxE* paralogues seems crucial for the maintenance of neural crest identity. Interestingly, it has recently been shown that *Sox10* alone is sufficient to reprogram fibroblast cells to a neural crest fate, highlighting the importance of *SoxE* genes in neural crest specification[74]. Furthermore, acquisition of migratory ability by the neural crest may have been fostered by diversification of receptors and ligands that enabled chemotactic behaviour. Genome-wide analysis shows that vertebrates have a much more complex arsenal of such molecules than do invertebrate chordates[58,75]. Thus, although the role of whole-genome duplications in neural crest evolution is still not fully understood, it

is likely that these duplications provided the neural crest with the molecular toolkit necessary for its complex behaviour.

Evolution of crest populations along the rostrocaudal axis

Neural crest cells arising from different levels of the neural axis are endowed with distinct developmental potentials and behaviour. For example, the cranial neural crest of gnathostomes gives rise to ectomesenchymal derivatives (for example, the bone and cartilage of the face) in addition to melanocytes, glia and a subset of cranial sensory neurons. By contrast, the trunk neural crest is not able to contribute to cartilage or bone *in vivo*. Rather, these cells form melanocytes, dorsal root and sympathetic ganglia and chromaffin cells. Although the gene regulatory interactions underlying these differences remain unknown, they probably reflect disparities in the mechanisms of specification observed among neural crest subpopulations[33].

Classic heterotopic grafting experiments in the chick demonstrate that the trunk neural crest has restricted developmental potential compared with the cranial population (reviewed in ref. 4). Cranial neural crest cells transplanted to the trunk can not only give rise to all trunk neural crest derivatives, but also form ectopic cartilage nodules that are characteristic of their site of origin[76,77]. By contrast, trunk neural crest transplanted to the head fail to contribute to facial bone and cartilage, although they can form sensory neurons and glia[78]. These results indicate that there are cell-autonomous differences between neural crest subpopulations established during specification. This is consistent with *cis*-regulatory analysis of neural-crest-specifier genes, which shows that expression of both *FoxD3* and *Sox10* in the neural crest is controlled by separate enhancers in the head compared with the trunk[33,34]. Furthermore, activity of these enhancers depends on axial-specific inputs, suggesting that specification of the cranial and trunk neural crest cells relies on different genetic programs[33,38].

The potential of the trunk neural crest has important implications for vertebrate evolution. For instance, it has been suggested that the neural crest played a central part in gnathostome evolution by giving rise to the exoskeleton of early vertebrates such as ostracoderms (armoured fishes)[41]. According to this scenario, at some point during vertebrate evolution the trunk neural crest was endowed with ectomesenchymal potential, which was subsequently lost in extant vertebrates. This hypothesis is based mainly on the fact that the skeletal plates that form the exoskeleton in armoured fishes were composed of dentine, a bona fide neural crest derivative[79,80]. Furthermore, studies in different model organisms suggest that the trunk neural crest exhibits at least some ectomesenchymal potential. For example, fate-map studies in zebrafish and frogs using vital dyes indicate that trunk neural crest contributes to the mesenchyme of the fins[80,81]. Finally, *in vitro* clonal analysis of avian trunk neural crest cells has shown that some clones exhibit gene expression that is characteristic of cartilage and bone[82], suggesting that these cells might possess a latent ectomesenchymal potential, which can be unlocked by environmental signals[83]. These studies suggest that the trunk neural crest might have some residual capacity to form ectomesenchyme, consistent with the hypothesis that the trunk neural crest gave rise to the exoskeleton of basal gnathostomes.

Recently, however, this view has been challenged by a number of studies that employ genetic fate mapping and cell-transplantation analysis to define neural crest contributions in teleost fishes (Box 4). These data show that mesenchyme-derived structures formerly attributed to the trunk neural crest lineage, such as the fin osteoblast, fin mesenchyme and mineral-forming cells of the scales, are in fact of mesodermal origin[84–87]. Taken together, these studies suggest that the trunk neural crest of teleosts has the same developmental restrictions observed in amniotes, calling into question the neural crest origin of the exoskeleton in armoured fishes. Although further studies in other model organisms are necessary for a pan-vertebrate view of trunk neural crest potential, these results indicate that trunk neural crest has been devoid of skeletogenic potential throughout its evolutionary history. These findings suggest that alternative hypotheses for the evolution of the neural crest subpopulations require consideration.

A second scenario is that the cranial neural crest was endowed with gene regulatory mechanisms that are absent from the trunk and may have been 'added on' early in vertebrate evolution. So far, a few developmentally important cranial-specific regulators have been identified. In gnathostomes, for example, *Ets1* (ref. 88) and *Id2* (ref. 89) are enriched in cranial crest cells and are crucial neural-crest-specifier genes for this subpopulation, but their expression is absent from the trunk. This raises the intriguing possibility that the genetic circuits underlying ectomesenchymal potential were added to an ancestral, trunk-like neural crest GRN. According to this view, the ectomesenchymal machinery was either co-opted from the mesoderm[26] or assembled *de novo* in the cranial region. This scenario implies that trunk neural crest cells have a simpler GRN topology than cranial neural crest, an experimentally tractable hypothesis that can be addressed by comparative studies. This view is consistent with the large number of transcriptional regulators that are shared among all neural crest populations, consistent with a common origin.

However, a complication is that transcription of genes such as *Sox10* and *FoxD3* is activated uniformly along the entire neural axis, but by distinct enhancers with differential inputs in the trunk compared with cranial regions[33,34]. A third scenario is that neural crest subpopulations may have segregated early in vertebrate evolution and possess different GRN topology. Consistent with enhancer analysis, this hypothesis suggests that many ancestral neural crest GRN connections have been rewired during evolution and that these changes in topology resulted in two populations that have multiple differences in potential and behaviour, despite sharing a similar genetic toolbox. This scenario implies that the trunk and cranial neural crest GRNs have substantial differences, and predicts that pan-neural crest genes are generally controlled by distinct, axial-specific enhancers. Importantly, the hypotheses already discussed can be tested by in-depth analysis of the genetic pathways controlling neural crest formation at different axial levels. In particular, elucidating the circuits controlling ectomesenchymal differentiation of the neural crest will have a great effect on how we interpret the evolution of this cell population. Furthermore, additional neural crest subpopulations exist, including vagal and sacral subtypes, which have distinct migratory pathways and contribute to different derivatives. A more inclusive gene regulatory view of these subpopulations might clarify how the developmental potential of the neural crest

BOX 4

Dermal skeleton

A dermal skeleton derived from odontodes is present in many vertebrates, both fossil and living. Dermal skeletal elements among living vertebrates include fin rays (lepidotrichia) of ray-finned (actinoptyerygian) fishes and scales, with multiple subtypes including placoid, ganoid and elasmoid scales in various taxa. Dermal skeletal elements have been proposed to be neural-crest-derived[122] at both cranial and trunk levels. However, recent analyses indicate that osteoblasts responsible for the elasmoid integumentary scales and fin rays of zebrafish derive from mesenchyme of mesodermal origin[87] rather than neural crest[80,123]. Similarly, ossified turtle shells that had been suggested to originate from both mesoderm-derived (endochondral rib) and neural-crest-derived (dermal) osteocytes, instead seem to develop only from mesoderm[124]. These data raise the question of whether the extensive dermal armour of stem gnathostomes originated from mesoderm or neural crest. At trunk levels, these dermal plates may have originated from mesoderm rather than neural crest, although they do arise from neural crest at cranial levels. However, it remains possible that neural crest cells contribute to other scale types, including the placoid scales of cartilaginous fishes that some have argued are more similar to dermal armour of early fishes[87].

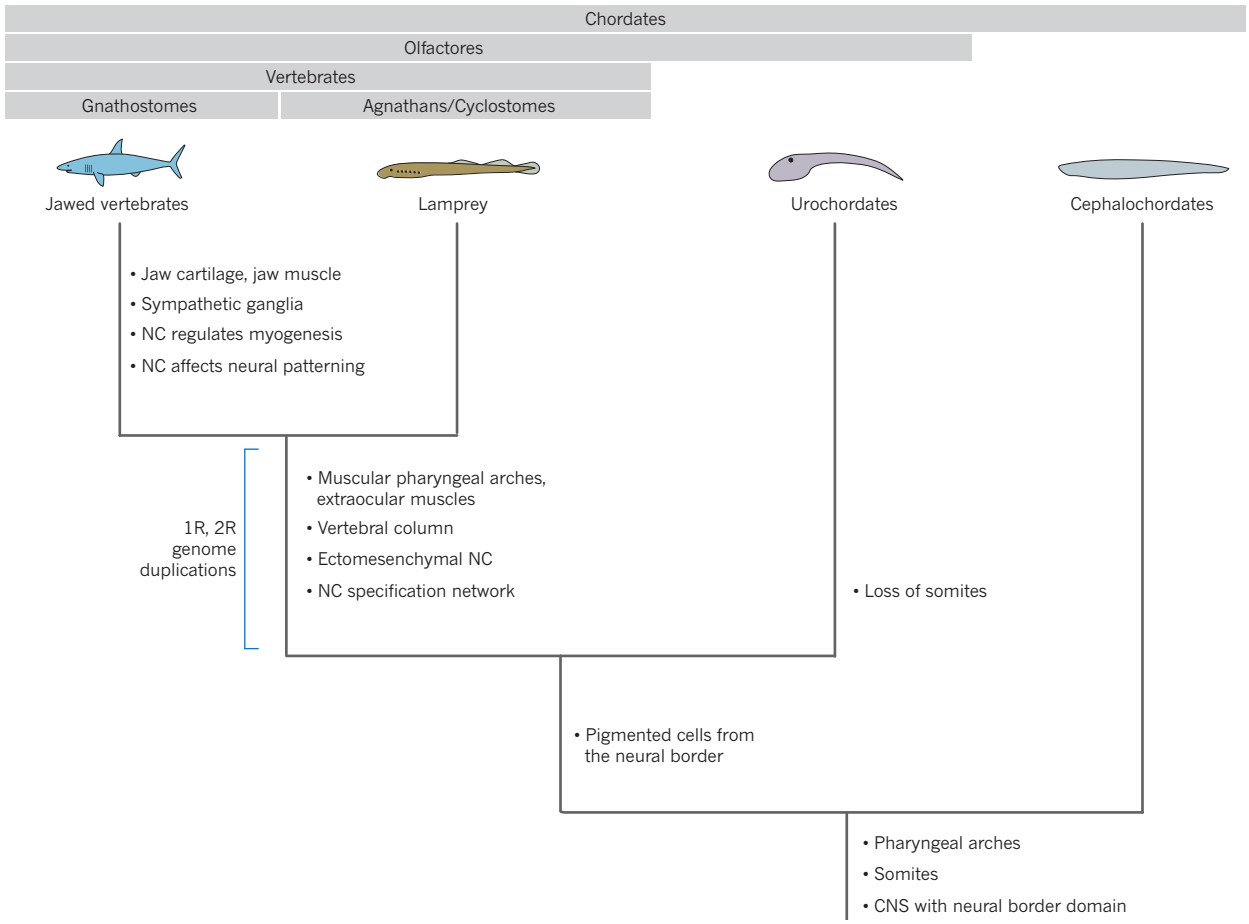


Figure 2 | Schematic cladogram of chordate features associated with neural crest cells or their derivatives. Labels at top indicate names of monophyletic groupings below. The timing of duplications is indicated in blue, whereas character changes are indicated by the bullet points. The order of character changes within a stem group is arbitrary. Adapted from ref. 97. CNS, central nervous system; NC, neural crest.

is established at the regulatory level, and have an impact on our views of the evolution of the vertebrate body plan.

Adult neural crest stem cells and post-embryonic growth

Many fossils suggest that the body size of the earliest vertebrates was, like many living invertebrates, quite small[90]. Only later did vertebrates begin to attain larger sizes, presumably through a process that involved extending the duration of post-embryonic growth. Extended growth requires coordinated development of many cell types, possibly including the establishment of stem-cell niches that govern the growth and regeneration of novel tissues.

Until recently, there was little indication of how adult neural crest cell populations were maintained. Recent evidence suggests that amniotes have adult neural crest stem-cell populations that maintain multipotency into adulthood, and which might enable the continuous replenishment of neural-crest-derived tissues[91,92], thus facilitating post-embryonic growth in concert with other tissues. These cells, called Schwann-cell precursors, reside on peripheral nerves and can produce multiple derivatives, including pigment cells and parasympathetic ganglia[93–96]. Whether the GRN underlying differentiation of these neural crest stem cells mirrors that of embryonic progenitor cells is an open and intriguing question that warrants further study. So far, these cells have only been identified in amniotes (in mammals and avians), but there is an obvious need for cells that fill this requirement in other vertebrates, and it is likely that cells such as these originated in early vertebrates.

These studies suggest that the influence of the neural crest in moulding the vertebrate body plan may extend beyond embryonic development, perhaps influencing the increase in size observed in several vertebrate clades. As vertebrates continued to grow post-embryonically, they may have required the setting aside of a population of neural crest stem cells, in the form of Schwann-cell precursors, that were retained to later stages. The relative proportion of adult tissues that these crest-derived stem cells contribute to is not yet known. Emerging data suggest that this cell population may form many derivatives classically attributed to the embryonic neural crest. Equally, they may represent the key to post-embryonic growth of the vertebrate body and therefore play a heretofore unknown part in promoting vertebrate evolution.

Expansion of neural crest cell types

Development of the neural crest sets vertebrates apart from invertebrate chordates. Formation of this novel cell type was probably facilitated by the addition of a new and uniquely vertebrate 'specification' kernel to the GRN, which in turn conferred multipotency and migratory ability to cells at the neural plate border. During the course of vertebrate evolution, even more derivatives have emerged under the umbrella of the neural crest (for example, additional elements to the peripheral nervous system, elaboration of the jaw or formation of the middle ear). Consolidation of key neural crest specifier genes such as *FoxD3*, *SoxE* and *TFAP2* in the neural-crest-specification module of its GRN may have facilitated evolution of this cell type, by allowing co-option of additional differentiation batteries under the control of neural crest regulators. Arguably, this has made the neural crest one of the most rapidly changing cell types in the vertebrate embryo and has perhaps contributed to the maintenance of neural crest stem cells in adults. ■

Received 10 September 2014; accepted 5 February 2015.

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

23 APRIL 2015 | VOL 520 | NATURE | 480

2. Northcutt, R. G. The new head hypothesis revisited. *J. Exp. Zool. B Mol. Dev. Evol.* **304,** 274–297 (2005).
This article discusses the new head hypothesis in light of more recent data.
3. Gee, H. *Before the Backbone: Views on the Origin of the Vertebrates* (Chapman & Hall, 1996).
4. Le Douarin, N. & Kalcheim, C. *The Neural Crest* (Cambridge Univ. Press, 1999).
5. Patthey, C., Schlosser, G. & Shimeld, S. M. The evolutionary history of vertebrate cranial placodes — I: cell type evolution. *Dev. Biol.* **389,** 82–97 (2014).
6. Schlosser, G., Patthey, C. & Shimeld, S. M. The evolutionary history of vertebrate cranial placodes — II. Evolution of ectodermal patterning. *Dev. Biol.* **389,** 98–119 (2014).
7. Hall, B. K. The neural crest as a fourth germ layer and vertebrates as quadroblastic not triploblastic. *Evol. Dev.* **2,** 3–5 (2000).
8. Sauka-Spengler, T. & Bronner-Fraser, M. Evolution of the neural crest viewed from a gene regulatory perspective. *Genesis* **46,** 673–682 (2008).
9. Holland, N. D. & Chen, J. Origin and early evolution of the vertebrates: new insights from advances in molecular biology, anatomy, and palaeontology. *Bioessays* **23,** 142–151 (2001).
10. Sauka-Spengler, T., Meulemans, D., Jones, M. & Bronner-Fraser, M. Ancient evolutionary origin of the neural crest gene regulatory network. *Dev. Cell* **13,** 405–420 (2007).
This work demonstrated that the lamprey has neural crest GRN components that are homologous to those in other vertebrates in both expression pattern and function, indicating that the neural crest GRN is largely shared throughout all vertebrates.
11. Bronner-Fraser, M. & Fraser, S. E. Cell lineage analysis reveals multipotency of some avian neural crest cells. *Nature* **335,** 161–164 (1988).
12. Bronner-Fraser, M. & Fraser, S. Developmental potential of avian trunk neural crest cells *in situ*. *Neuron* **3,** 755–766 (1989).
13. Frank, E. & Sanes, J. R. Lineage of neurons and glia in chick dorsal root ganglia: analysis *in vivo* with a recombinant retrovirus. *Development* **111,** 895–908 (1991).
14. Dupin, E., Calloni, G. W. & Le Douarin, N. M. The cephalic neural crest of amniote vertebrates is composed of a large majority of precursors endowed with neural, melanocytic, chondrogenic and osteogenic potentialities. *Cell Cycle* **9,** 238–249 (2010).
15. Calloni, G. W., Le Douarin, N. M. & Dupin, E. High frequency of cephalic neural crest cells shows coexistence of neurogenic, melanogenic, and osteogenic differentiation capacities. *Proc. Natl Acad. Sci. USA* **106,** 8947–8952 (2009).
16. Heimberg, A. M., Cowper-Sal-lari, R., Sémon, M., Donoghue, P. C. J. & Peterson, K. J. microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. *Proc. Natl Acad. Sci. USA* **107,** 19379–19383 (2010).
17. Donoghue, P. C. J. & Keating, J. N. Early vertebrate evolution. *Palaeontology* **57,** 879–893 (2014).
18. Oisi, Y., Ota, K. G., Kuraku, S., Fujimoto, S. & Kuratani, S. Craniofacial development of hagfishes and the evolution of vertebrates. *Nature* **493,** 175–180 (2013).
19. Ota, K. G. & Kuratani, S. Cyclostome embryology and early evolutionary history of vertebrates. *Integr. Comp. Biol.* **47,** 329–337 (2007).
20. Shimeld, S. M. & Donoghue, P. C. J. Evolutionary crossroads in developmental biology: cyclostomes (lamprey and hagfish). *Development* **139,** 2091–2099 (2012).
21. Hall, B. K. & Gillis, J. A. Incremental evolution of the neural crest, neural crest cells and neural crest-derived skeletal tissues. *J. Anat.* **222,** 19–31 (2013).
22. Mongera, A. *et al*. Genetic lineage labeling in zebrafish uncovers novel neural crest contributions to the head, including gill pillar cells. *Development* **140,** 916–925 (2013).
This paper identifies gill pillar cells, which are crucial for gill structure throughout vertebrates, as neural crest derivatives.
23. Häming, D. *et al*. Expression of sympathetic nervous system genes in lamprey suggests their recruitment for specification of a new vertebrate feature. *PLoS ONE* **6,** e26543 (2011).
24. Medeiros, D. M. The evolution of the neural crest: new perspectives from lamprey and invertebrate neural crest-like cells. *Wiley Interdiscip. Rev. Dev. Biol.* **2,** 1–15 (2013).
25. Meulemans, D. & Bronner-Fraser, M. Central role of gene cooption in neural crest evolution. *J. Exp. Zool. B. Mol. Dev. Evol.* **304,** 298–303 (2005).
26. Jandzik, D. *et al*. Evolution of the new vertebrate head by co-option of an ancient chordate skeletal tissue. *Nature* **518,** 534–537 (2015).
This crucial paper identifies cellular cartilage in a cephalochordate, lending support to the contention that neural-crest-derived cartilage was co-opted from other tissues rather than constructed *de novo*.
27. Davidson, E. H. *The Regulatory Genome* (Academic, 2010).
28. Erwin, D. H. & Davidson, E. H. The evolution of hierarchical gene regulatory networks. *Nature Rev. Genet.* **10,** 141–148 (2009).
29. de Crozé, N., Maczkowiak, F. & Monsoro-Burq, A. H. Reiterative AP2a activity controls sequential steps in the neural crest gene regulatory network. *Proc. Natl Acad. Sci. USA* **108,** 155–160 (2011).
30. Nieto, M. A., Sargent, M. G., Wilkinson, D. G. & Cooke, J. Control of cell behavior during vertebrate development by *Slug*, a zinc finger gene. *Science* **264,** 835–839 (1994).
31. Labosky, P. A. & Kaestner, K. H. The winged helix transcription factor Hfh2 is expressed in neural crest and spinal cord during mouse development. *Mech. Dev.* **76,** 185–190 (1998).
32. Dottori, M., Gross, M. K., Labosky, P. & Goulding, M. The winged-helix transcription factor Foxd3 suppresses interneuron differentiation and promotes neural crest cell fate. *Development* **128,** 4127–4138 (2001).
33. Simões-Costa, M. S., McKeown, S. J., Tan-Cabugao, J., Sauka-Spengler, T. & Bronner, M. E. Dynamic and differential regulation of stem cell factor FoxD3 in the neural crest is encrypted in the genome. *PLoS Genet.* **8,** e1003142 (2012).
34. Betancur, P., Bronner-Fraser, M. & Sauka-Spengler, T. Genomic code for Sox10 activation reveals a key regulatory enhancer for cranial neural crest. *Proc. Natl Acad. Sci. USA* **107,** 3570–3575 (2010).
35. McKeown, S. J., Lee, V. M., Bronner-Fraser, M., Newgreen, D. F. & Farlie, P. G. Sox10 overexpression induces neural crest-like cells from all dorsoventral levels of the neural tube but inhibits differentiation. *Dev. Dyn.* **233,** 430–444 (2005).
36. Meulemans, D. & Bronner-Fraser, M. Gene-regulatory interactions in neural crest evolution and development. *Dev. Cell* **7,** 291–299 (2004).
37. Betancur, P., Bronner-Fraser, M. & Sauka-Spengler, T. Assembling neural crest regulatory circuits into a gene regulatory network. *Annu. Rev. Cell Dev. Biol.* **26,** 581–603 (2010).
38. Simões-Costa, M. & Bronner, M. E. Establishing neural crest identity: a gene regulatory recipe. *Development* **142,** 242–257 (2015).
39. Sauka-Spengler, T. & Bronner-Fraser, M. A gene regulatory network orchestrates neural crest formation. *Nature Rev. Mol. Cell Biol.* **9,** 557–568 (2008).
40. Simões-Costa, M. & Bronner, M. E. Insights into neural crest development and evolution from genomic analysis. *Genome Res.* **23,** 1069–1080 (2013).
41. Donoghue, P. C. J., Graham, A. & Kelsh, R. N. The origin and evolution of the neural crest. *Bioessays* **30,** 530–541 (2008).
42. Meulemans, D. & Bronner-Fraser, M. Amphioxus and lamprey AP-2 genes: implications for neural crest evolution and migration patterns. *Development* **129,** 4953–4962 (2002).
43. Ota, K. G., Kuraku, S. & Kuratani, S. Hagfish embryology with reference to the evolution of the neural crest. *Nature* **446,** 672–675 (2007).
44. Aybar, M. J., Nieto, M. A. & Mayor, R. *Snail* precedes *Slug* in the genetic cascade required for the specification and migration of the *Xenopus* neural crest. *Development* **130,** 483–494 (2003).
45. LaBonne, C. & Bronner-Fraser, M. Snail-related transcriptional repressors are required in *Xenopus* for both the induction of the neural crest and its subsequent migration. *Dev. Biol.* **221,** 195–205 (2000).
46. Oram, K. F. & Gridley, T. Mutations in Snail family genes enhance craniosynostosis of *Twist1* haplo-insufficient mice: implications for Saethre-Chotzen Syndrome. *Genetics* **170,** 971–974 (2005).
47. Rogers, C. D., Saxena, A. & Bronner, M. E. Sip1 mediates an E-cadherin-to-N-cadherin switch during cranial neural crest EMT. *J. Cell Biol.* **203,** 835–847 (2013).
48. Jeffery, W. R. *et al*. Trunk lateral cells are neural crest-like cells in the ascidian *Ciona intestinalis*: insights into the ancestry and evolution of the neural crest. *Dev. Biol.* **324,** 152–160 (2008).
49. Abitua, P. B., Wagner, E., Navarrete, I. A. & Levine, M. Identification of a rudimentary neural crest in a non-vertebrate chordate. *Nature* **492,** 104–107 (2012).
This paper argues that a gene regulatory network acting in the *C. intestinalis* a9.49 cell lineage is homologous to the GRN of vertebrate neural crest, and suggests that co-option of mesenchymal migration controls might have facilitated expansion of neural crest derivatives in early vertebrates.
50. Ivashkin, E. & Adameyko, I. Progenitors of the protochordate ocellus as an evolutionary origin of the neural crest. *Evodevo* **4,** 12 (2013).
51. Yu, J.-K., Meulemans, D., McKeown, S. J. & Bronner-Fraser, M. Insights from the amphioxus genome on the origin of vertebrate neural crest. *Genome Res.* **18,** 1127–1132 (2008).
This paper showed that an AmphiFoxD regulatory element was capable of driving expression in chicken somites, but not in neural crest, suggesting that novel regulatory elements were required for AmphiFoxD incorporation into the neural crest GRN.
52. Langeland, J. A., Tomsa, J. M., Jackman, W. R. & Kimmel, C. B. An amphioxus snail gene: expression in paraxial mesoderm and neural plate suggests a conserved role in patterning the chordate embryo. *Dev. Genes Evol.* **208,** 569–577 (1998).
53. Denes, A. S. *et al*. Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* **129,** 277–288 (2007).
54. Ohno, S. *Evolution by Gene Duplication* (Springer, 1970).
55. Yu, J.-K., Holland, N. D. & Holland, L. Z. An amphioxus winged helix/forkhead gene, *AmphiFoxD*: insights into vertebrate neural crest evolution. *Dev. Dyn.* **225,** 289–297 (2002).
56. Yu, J.-K. S. The evolutionary origin of the vertebrate neural crest and its developmental gene regulatory network — insights from amphioxus. *Zoology (Jena)* **113,** 1–9 (2010).
57. Parker, H. J., Bronner, M. E. & Krumlauf, R. A Hox regulatory network of hindbrain segmentation is conserved to the base of vertebrates. *Nature* **514,** 490–493 (2014).
58. Dehal, P. The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* **298,** 2157–2167 (2002).
59. Holland, L. Z. *et al*. The amphioxus genome illuminates vertebrate origins and cephalochordate biology. *Genome Res.* **18,** 1100–1111 (2008).
60. Yu, J.-K., Holland, N. D. & Holland, L. Z. Tissue-specific expression of FoxD reporter constructs in amphioxus embryos. *Dev. Biol.* **274,** 452–461 (2004).
61. Peter, I. S. & Davidson, E. H. Evolution of gene regulatory networks controlling body plan development. *Cell* **144,** 970–985 (2011).
62. Ono, H., Kozmik, Z., Yu, J.-K. & Wada, H. A novel N-terminal motif is responsible for the evolution of neural crest-specific gene-regulatory activity in vertebrate FoxD3. *Dev. Biol.* **385,** 396–404 (2014).

63. Taylor, J. S. & Raes, J. Duplication and divergence: the evolution of new genes and old ideas. *Annu. Rev. Genet.* **38,** 615–643 (2004).
64. Vandepoele, K., De Vos, W., Taylor, J. S., Meyer, A. & Van de Peer, Y. Major events in the genome evolution of vertebrates: paranome age and size differ considerably between ray-finned fishes and land vertebrates. *Proc. Natl Acad. Sci. USA* **101,** 1638–1643 (2004).
65. Crow, K. D., Wagner, G. P. & SMBE Tri-National Young Investigators. Proceedings of the SMBE Tri-National Young Investigators' Workshop 2005. What is the role of genome duplication in the evolution of complexity and diversity? *Mol. Biol. Evol.* **23,** 887–892 (2006).
66. Holland, P. W., Garcia-Fernàndez, J., Williams, N. A. & Sidow, A. Gene duplications and the origins of vertebrate development. *Dev. Suppl.* **1994,** 125–133 (1994).
67. Holland, L. Z. Evolution of new characters after whole genome duplications: insights from amphioxus. *Semin. Cell Dev. Biol.* **24,** 101–109 (2013).
68. Smith, J. J. *et al.* Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. *Nature Genet.* **45,** 415–421 (2013).
69. Kuraku, S. Insights into cyclostome phylogenomics: pre-2R or post-2R. *Zoolog. Sci.* **25,** 960–968 (2008).
70. Kuraku, S., Meyer, A. & Kuratani, S. Timing of genome duplications relative to the origin of the vertebrates: did cyclostomes diverge before or after? *Mol. Biol. Evol.* **26,** 47–59 (2009).
71. Smith, J. J. The sea lamprey meiotic map resolves ancient vertebrate genome duplications. Preprint at http://dx.doi.org/10.1101/008953 (2014).
72. Carroll, S. B. Evolution at two levels: on genes and form. *PLoS Biol.* **3,** e245 (2005).
73. Kassahn, K. S., Dang, V. T., Wilkins, S. J., Perkins, A. C. & Ragan, M. A. Evolution of gene function and regulatory control after whole-genome duplication: comparative analyses in vertebrates. *Genome Res.* **19,** 1404–1418 (2009).
74. Kim, Y. J. *et al.* Generation of multipotent induced neural crest by direct reprogramming of human postnatal fibroblasts with a single transcription factor. *Cell Stem Cell* **15,** 497–506 (2014).
75. Emes, R. D. *et al.* Evolutionary expansion and anatomical specialization of synapse proteome complexity. *Nature Neurosci.* **11,** 799–806 (2008).
76. Le Douarin, N. M. & Teillet, M. A. Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neurectodermal mesenchymal derivatives, using a biological cell marking technique. *Dev. Biol.* **41,** 162–184 (1974).
77. Le Lièvre, C. S., Schweizer, G. G., Ziller, C. M. & Le Douarin, N. M. Restrictions of developmental capabilities in neural crest cell derivatives as tested by *in vivo* transplantation experiments. *Dev. Biol.* **77,** 362–378 (1980).
78. Le Lièvre, C. S. & Le Douarin, N. M. Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. *J. Embryol. Exp. Morphol.* **34,** 125–154 (1975).
79. Sire, J.-Y., Donoghue, P. C. J. & Vickaryous, M. K. Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *J. Anat.* **214,** 409–440 (2009).
80. Smith, M., Hickman, A., Amanze, D., Lumsden, A. & Thorogood, P. Trunk neural crest origin of caudal fin mesenchyme in the zebrafish *Brachydanio rerio*. *Proc. R. Soc. Lond. B* **256,** 137–145 (1994).
81. Collazo, A., Bronner-Fraser, M. & Fraser, S. E. Vital dye labelling of *Xenopus laevis* trunk neural crest reveals multipotency and novel pathways of migration. *Development* **118,** 363–376 (1993).
82. Coelho-Aguiar, J. M., Le Douarin, N. M. & Dupin, E. Environmental factors unveil dormant developmental capacities in multipotent progenitors of the trunk neural crest. *Dev. Biol.* **384,** 13–25 (2013).
83. McGonnell, I. M. & Graham, A. Trunk neural crest has skeletogenic potential. *Curr. Biol.* **12,** 767–771 (2002).
84. Lee, R. T. H., Knapik, E. W., Thiery, J. P. & Carney, T. J. An exclusively mesodermal origin of fin mesenchyme demonstrates that zebrafish trunk neural crest does not generate ectomesenchyme. *Development* **140,** 2923–2932 (2013).
This recent paper finds that neural crest cells at trunk levels do not contribute to fin mesenchyme, in contrast to earlier claims.
85. Lee, R. T. H., Thiery, J. P. & Carney, T. J. Dermal fin rays and scales derive from mesoderm, not neural crest. *Curr. Biol.* **23,** R336–R337 (2013).
86. Shimada, A. *et al.* Trunk exoskeleton in teleosts is mesodermal in origin. *Nature Commun.* **4,** 1639 (2013).
This paper suggests that mesoderm, and not neural crest, gives rise to the exoskeleton at trunk levels, conflicting with findings of earlier studies and calling into question whether trunk neural crest has ectomesenchymal capability.
87. Mongera, A. & Nüsslein-Volhard, C. Scales of fish arise from mesoderm. *Curr. Biol.* **23,** R338–R339 (2013).
88. Théveneau, E., Duband, J.-L. & Altabef, M. Ets-1 confers cranial features on neural crest delamination. *PLoS ONE* **2,** e1142 (2007).
89. Martinsen, B. J. & Bronner-Fraser, M. Neural crest specification regulated by the helix-loop-helix repressor Id2. *Science* **281,** 988–991 (1998).
90. Janvier, P. *Early Vertebrates* (Oxford Univ. Press, 1996).
91. Jinno, H. *et al.* Convergent genesis of an adult neural crest-like dermal stem cell from distinct developmental origins. *Stem Cells* **28,** 2027–2040 (2010).
92. Morrison, S. J., White, P. M., Zock, C. & Anderson, D. J. Prospective identification, isolation by flow cytometry, and *in vivo* self-renewal of multipotent mammalian neural crest stem cells. *Cell* **96,** 737–749 (1999).
93. Dyachuk, V. *et al.* Neurodevelopment. Parasympathetic neurons originate from nerve-associated peripheral glial progenitors. *Science* **345,** 82–87 (2014).
One of two papers demonstrating that Schwann-cell precursors also give rise to parasympathetic neurons.
94. Espinosa-Medina, I. *et al.* Neurodevelopment. Parasympathetic ganglia derive from Schwann cell precursors. *Science* **345,** 87–90 (2014).
The second of two papers demonstrating that Schwann-cell precursors are a source of parasympathetic neurons.
95. Adameyko, I. *et al.* Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. *Cell* **139,** 366–379 (2009).
This paper identifies Schwann-cell precursors as a major source of pigment cells in chicken and mouse.
96. Krause, M. P. *et al.* Direct genesis of functional rodent and human Schwann cells from skin mesenchymal precursors. *Stem Cell Rep.* **3,** 85–100 (2014).
97. Green, S. A. & Bronner, M. E. The lamprey: a jawless vertebrate model system for examining origin of the neural crest and other vertebrate traits. *Differentiation* **87,** 44–51 (2014).
98. Northcutt, R. G. & Gans, C. The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Q. Rev. Biol.* **58,** 1–28 (1983).
99. Graham, A. Deconstructing the pharyngeal metamere. *J. Exp. Zool. B Mol. Dev. Evol.* **310,** 336–344 (2008).
100. McCauley, D. W. & Bronner-Fraser, M. Neural crest contributions to the lamprey head. *Development* **130,** 2317–2327 (2003).
101. Gillis, J. A., Fritzenwanker, J. H. & Lowe, C. J. A stem-deuterostome origin of the vertebrate pharyngeal transcriptional network. *Proc. Biol. Sci.* **279,** 237–246 (2012).
102. Smith, A. B. The pre-radial history of echinoderms. *Geolog. J.* **40,** 255–280 (2005).
103. Graham, A. & Richardson, J. Developmental and evolutionary origins of the pharyngeal apparatus. *Evodevo* **3,** 24 (2012).
104. Yasui, K., Kaji, T., Morov, A. R. & Yonemura, S. Development of oral and branchial muscles in lancelet larvae of *Branchiostoma japonicum*. *J. Morphol.* **275,** 465–477 (2014).
105. Trinajstic, K. *et al.* Fossil musculature of the most primitive jawed vertebrates. *Science* **341,** 160–164 (2013).
106. Matsuoka, T. *et al.* Neural crest origins of the neck and shoulder. *Nature* **436,** 347–355 (2005).
107. Köntges, G. & Lumsden, A. Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* **122,** 3229–3242 (1996).
108. Fraser, G. J., Cerny, R., Soukup, V., Bronner-Fraser, M. & Streelman, J. T. The odontode explosion: the origin of tooth-like structures in vertebrates. *Bioessays* **32,** 808–817 (2010).
109. Murdock, D. J. E. *et al.* The origin of conodonts and of vertebrate mineralized skeletons. *Nature* **502,** 546–549 (2013).
110. Janvier, P. Inside-out turned upside-down. *Nature* **502,** 457–458 (2013).
111. Creuzet, S. E., Martinez, S. & Le Douarin, N. M. The cephalic neural crest exerts a critical effect on forebrain and midbrain development. *Proc. Natl Acad. Sci. USA* **103,** 14033–14038 (2006).
112. Le Douarin, N. M., Couly, G. & Creuzet, S. E. The neural crest is a powerful regulator of pre-otic brain development. *Dev. Biol.* **366,** 74–82 (2012).
113. Aguiar, D. P., Sghari, S. & Creuzet, S. The facial neural crest controls fore- and midbrain patterning by regulating Foxg1 expression through Smad1 activity. *Development* **141,** 2494–2505 (2014).
114. Holland, L. Z. *et al.* Evolution of bilaterian central nervous systems: a single origin? *Evodevo* **4,** 27 (2013).
115. Pani, A. M. *et al.* Ancient deuterostome origins of vertebrate brain signalling centres. *Nature* **483,** 289–294 (2012).
116. Noden, D. M. & West, P. F. The differentiation and morphogenesis of craniofacial muscles. *Dev. Dyn.* **235,** 1194–1218 (2006).
117. Sambasivan, R., Kuratani, S. & Tajbakhsh, S. An eye on the head: the development and evolution of craniofacial muscles. *Development* **138,** 2401–2415 (2011).
118. Lee, G.-H., Chang, M.-Y., Hsu, C.-H. & Chen, Y.-H. Essential roles of basic helix-loop-helix transcription factors, Capsulin and Musculin, during craniofacial myogenesis of zebrafish. *Cell. Mol. Life Sci.* **68,** 4065–4078 (2011).
119. Tzahor, E. Heart and craniofacial muscle development: a new developmental theme of distinct myogenic fields. *Dev. Biol.* **327,** 273–279 (2009).
120. Kelly, R. G. Core issues in craniofacial myogenesis. *Exp. Cell Res.* **316,** 3034–3041 (2010).
121. Johnels, A. G. On the peripheral autonomic nervous system of the trunk region of *Lampetra planeri*. *Acta Zool.* **37,** 251–286 (1956).
122. Donoghue, P. C. J. & Sansom, I. J. Origin and early evolution of vertebrate skeletonization. *Microsc. Res. Tech.* **59,** 352–372 (2002).
123. Kague, E. *et al.* Skeletogenic fate of zebrafish cranial and trunk neural crest. *PLoS ONE* **7,** e47394 (2012).
124. Hirasawa, T., Nagashima, H. & Kuratani, S. The endoskeletal origin of the turtle carapace. *Nature Commun.* **4,** 2107 (2013).

Acknowledgements We would like to thank H. Parker, C. Rogers and L. Kerosuo for their comments and helpful discussion on this manuscript. This work was supported by National Institutes of Health (NIH) grant R01NS086907. M.S.-C. was funded by a fellowship from the Pew Foundation and by NIH grant 1K99DE024232.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this paper at go.nature.com/ss8k2k. Correspondence should be addressed to M.E.B. (mbronner@caltech.edu).

REVIEW

doi:10.1038/nature14437

Facts and fancies about early fossil chordates and vertebrates

Philippe Janvier[1]

The interrelationships between major living vertebrate, and even chordate, groups are now reasonably well resolved thanks to a large amount of generally congruent data derived from molecular sequences, anatomy and physiology. But fossils provide unexpected combinations of characters that help us to understand how the anatomy of modern groups was progressively shaped over millions of years. The dawn of vertebrates is documented by fossils that are preserved as either soft-tissue imprints, or minute skeletal fragments, and it is sometimes difficult for palaeontologists to tell which of them are reliable vertebrate remains and which merely reflect our idea of an ancestral vertebrate.

Vertebrates are a very small group among animals, but they show, along with arthropods and possibly echinoderms, a large number of 'fossilizable' complex characters that can be analysed to reconstruct their relationships; however, most of their anatomically informative fossil record appeared relatively late, about 470 million years ago (Ma). During the past 20 years or so, the fossil record of Palaeozoic era, 535–250 million year (Myr) old, jawless vertebrates has been enriched by the discovery of spectacular soft-bodied fossils preserved as imprints in famous fossil sites such as Chengjiang (535 Myr old) in China[1] and the Burgess Shale in Canada[2] (510 Myr old), but also in other, younger sites that yield exceptional preservation of soft tissues (referred to as 'Konservat-Lagerstätte'). These fossils, long considered to be trivial by palaeoanatomists, have gained a new dimension thanks to investigation and imaging techniques that allow the actual nature of the preserved tissues to be identified, as well as a better understanding of the processes involved in decay and fossilization, thereby avoiding their overinterpretation[3–6].

Palaeontologists have been extensively tracing the earliest evidence for typical vertebrate hard tissues, such as bone, calcified cartilage, dentine (the 'ivory' of our teeth) or enamel, generally in the form of bone fragments, isolated scales or denticles made up of bioapatite (calcium phosphate) and found scattered in early Palaeozoic sediments[7,8]. This search for vertebrate 'microremains' or 'ichthyoliths' (often the only available vertebrate remains in the early Palaeozoic) yielded a large diversity of skeletal elements that could be compared with those of previously known, younger, complete fossils that belong to the major vertebrate groups, and provided evidence for the antiquity of most classic vertebrate hard tissues at least since the Lower or Middle Ordovician (about 477 Ma). However, this research also yielded some skeletal elements that, although suggesting the shape of scales or teeth, do not show all the characteristics of hitherto recognized vertebrate hard tissues. Such cases are frequent among Ordovician to Silurian (480–420 Myr old) microremains, which are dismissed by some, but regarded as possible vertebrates by others. The vertebrate fossil record is documented by an abundance of articulated specimens from periods since the late Silurian (about 430 Ma), but is either poorly represented or very puzzling in earlier periods. However, late Silurian (430 Myr old) articulated vertebrates still turn up (in Scotland, Canada and China[9,10]), and hint at exciting issues in deeper vertebrate history.

This may give the reader the impression that the early history (before the late Silurian) of vertebrate evolution is documented by fossils that look rather like squashed slugs and crushed lobster carapaces, although sometimes articulated. Uninformative data indeed, but, practically, it is all we can offer, except for extremely rare three-dimensionally preserved jawless vertebrates, such as the Ordovician astraspids and arandaspids[11–13], which document the first occurrence of an extensive exoskeleton (or dermal skeleton, the superficial skeleton of vertebrates) with site-specific bones and a lateral-line system (the superficial sense organ of fishes).

Living vertebrates fall into two major clades, the cyclostomes (hagfishes and lampreys) and the gnathostomes (jawed vertebrates). Only the latter produce bone and dentine. Therefore, current vertebrate phylogenies that include fossils suggest that all the Palaeozoic jawless vertebrates that display at least an exoskeleton are more closely related to gnathostomes than to cyclostomes, and are thus 'stem gnathostomes', although lacking jaws[14]. These jawless stem gnathostomes that possess a calcified skeleton are informally referred to as 'ostracoderms' for historical reasons, but form

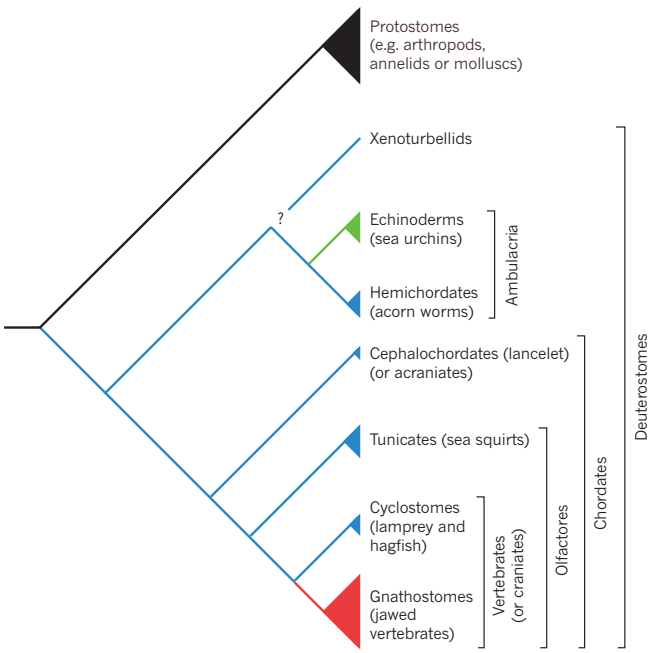


Figure 1 | Interrelationships of the major extant deuterostome clades. Distribution of the major tissues potentially preserved in fossil deuterostomes: no calcified hard tissue except for occasional calcified cartilage in vertebrates (blue), calcitic skeleton (green) and bone, dentine, enamel or enameloid (red).

[1]Muséum National d'Histoire Naturelle, UMR7207 du CNRS (Sorbonne Universités, UPMC), 8 rue Buffon, 75231 Paris, France.

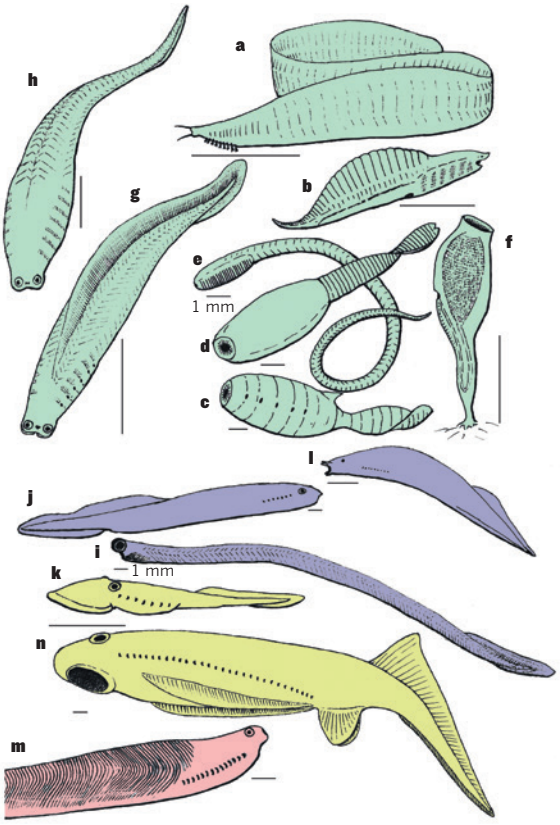


Figure 2 | Soft-bodied presumed fossil chordates and vertebrates, from the Cambrian (green), Silurian (pink), Devonian (yellow) and Carboniferous (purple) periods. **a**, *Pikaia* was long regarded as a chordate, but is now considered to be either of uncertain affinity, or possibly a close relative of yunnanozoans (adapted from ref. 22). **b**, The yunnanozoan *Haikouella* is a possible stem deuterostome or stem vertebrate (adapted from ref. 28). **c**, **d**, The vetulicolans *Didazoon* (**c**) and *Banffia* (**d**) are possible stem chordates, stem deuterostomes or stem protostomes (adapted from refs 31, 33). **e**, *Cathaymyrus* is a possible stem cephalochordate (adapted from ref. 34). **f**, *Shankouclava* is a likely tunicate (adapted from ref. 36). **g**, **h**, *Haikouichthys* (**g**) and *Metaspriggina* (**h**) are stem vertebrates (based on refs 40, 41). **i**, *Clydagnathus* is a euconodont (adapted from ref. 46). **j**, **k**, *Mayomyzon* (**j**) and *Priscomyzon* (**k**) are two fossil lampreys (adapted from refs 15, 17). **l**, *Myxinikela* is a probable hagfish (adapted from ref. 19). **m**, *Jamoytius* is a jawless stem gnathostome with thin mineralized body scales (adapted from ref. 60). **n**, *Euphanerops*, a jawless vertebrate whose calcified cartilage displays a lamprey-like annular cartilage and branchial basket (adapted from refs 61, 64). Scale bars are 10 mm (**a**–**d**, **f**–**h**, **j**–**n**) and 1 mm (**e**, **i**).

a grade: an array of groups that are more and more closely related to jawed vertebrates and whose anatomy documents the progressive assembly of the gnathostome body plan before the rise of jaws. By contrast, there is no evidence that cyclostomes have ever produced a mineralized skeleton, and neither the four fossil lampreys[15–18], nor the two possible fossil hagfishes[19,20] show any clear indication of a mineralized skeleton.

Soft-bodied chordates and wishful thinking

The bestiary of the Chengjiang and Burgess Shale sites[1,2] comprises a number of animals that have been referred to as either chordates or other deuterostome groups (Fig. 1). Most of these fossils have been referred to as chordates because they show at least some indication of either a notochord (the axial support of chordates, and precursor of the vertebral column), a segmented body structure or gill slits. Although the segmentation of the body musculature and gill apparatus has different developmental causes[21], it is often regarded as a 'signature' of the chordates, but is readily distinguished from the metamery (repeated parts) of arthropods or annelids. Notably, this was the case for *Pikaia* (Fig. 2a), from the Burgess Shale, whose body shows indications of a series of myomeres (muscle blocks) and a notochord, but whose head bears peculiar appendages (regarded as respiratory organs) and tentacles that are at odds with vertebrate anatomy[22]. Despite the exquisite preservation of numerous specimens of *Pikaia*, this long iconic 'vertebrate ancestor'[23] remains an enigma, and opinions about its affinities oscillate between the chordate hypothesis and a convergent morphology in some protostomes (the sister group of deuterostomes)[22] (Fig. 1). Yunnanozoans (*Yunnanozoon* and *Haikouella*; Fig. 2b) from Chengjiang have also been referred to as chordates[24] because of their presumed notochord, segmented body musculature covered by a cuticle and their seemingly vertebrate-like series of six gill pairs. Notably, they have been referred to as either stem deuterostomes[25], hemichordates, cephalochordates or stem vertebrates[26–28]. The controversy between the advocates of the stem-vertebrate[29] and stem-deuterostome[30] hypotheses reflects the difficulty in assessing the nature of the actual tissues and anatomical characters observed in these fossils. Vetulicolans[31,32] (*Vetulicola*, *Xidazoon*, *Didazoon* and *Pomatrum*; Fig. 2c) from Chengjiang and the somewhat similar *Banffia* (Fig. 2d) from the Burgess Shale display a bipartite structure, with a balloon-shaped, cuticle-covered head laterally pierced by five presumed gill openings, and a flattened segmented tail[33]. *Banffia*, however, seems devoid of gill openings and displays midgut diverticulae that rather suggest a protostome anatomy[33]. Again, the vetulicolan's gill openings might suggest a stem deuterostome, but the purported presence of an endostyle (a gland unique to chordates) suggests stem chordate affinity[32]. *Cathaymyrus* (Fig. 2e), from Chengjiang, was described as "*Pikaia*-like"[34]. It has a worm-shaped body with a long series of myomeres, and a distinct row of closely set pharyngeal slits that resemble those of cephalochordates. Other presumed chordates from Chengjiang are the debated tunicates *Cheungkongella*[35] and *Shankouclava*[36] (Fig. 2f). As a whole, all these presumed chordates from the Cambrian, mostly preserved as soft-tissue imprints, only provide tenuous information about their possible phylogenetic relationships. And, despite their often spectacular preservation, there is a risk of overinterpreting their anatomy in the light of widely different living organisms. A notable example of this problem is *Ainiktozoon* (a much younger fossil from the Silurian (430 Ma) of Scotland), which has been interpreted both as a possible chordate because of its segmented body[37] and as a thylacocephalan — a peculiar extinct arthropod group[38].

The myllokunmingiids (for example, *Myllokunmingia* and *Haikouichthys*; Fig. 2g)[39,40] from Chengjiang and the similar *Metaspriggina*[41] (Fig. 2h) from the Burgess Shale look more familiar to vertebrate specialists, as they are clearly 'fish-like'. Despite their similarities, *Metaspriggina* provides better information about the arrangement of gill bars and eye structure. Although only a small number of characters can actually be observed on this kind of material, character analyses have resolved myllokunmingiids as paraphyletic, with *Myllokunmingia* as a stem vertebrate, and *Haikouichthys* as a stem lamprey[39]. More recent analyses suggest that all myllokunmingiids, and probably *Metaspriggina*, are stem vertebrates, but appear in a basal polytomy in the vertebrate tree, more crownward than *Pikaia,* but less so than any crown-group vertebrate (the last common ancestor to living vertebrates and all their fossil relatives)[41]. By combining myllokunmingiids and *Metaspriggina* data, a better reconstruction of the most likely Cambrian vertebrates is possible — a jawless 'fish' with a pair of large, anterodorsally facing camera eyes, a small median olfactory organ, 5–7 pairs of gill arches, a stomach, a series of chevron-shaped myomeres and a median fin web (Fig. 2g, h), thereby remotely resembling old hypothetical reconstructions of ancestral vertebrates[42] (Box 1).

The soft-bodied fossil record of the vertebrates is not limited to the Cambrian, and after the Cambrian 'squashed slug' episode comes the saga of the conodonts. Conodonts are minute tooth- or comb-like elements, or denticles, that are made up of bioapatite (like vertebrate teeth) and occur in marine sediments from the Cambrian to the Late Triassic (about 530–200 Ma). Depending on their internal structure, conodonts fall into three groups: protoconodonts, paraconodonts and euconodonts, the latter being the only monophyletic one[43]. For more than a century, conodonts have received diverse, sometimes fanciful interpretations, until the 1983 publication of the first 'conodont-bearing animal', from the Carboniferous

(330 Ma): a conodont assemblage located in the mouth of an eel-shaped animal preserved as a soft-tissue imprint[44]. Other specimens have since turned up[45], but so far all known articulated conodont-bearing animals are euconodonts. Anatomically, a euconodont-bearing animal has a small head with large paired eyes, a mouth or pharynx containing a large number of denticles, an elongated eel-shaped body with chevron-shaped myomeres, and a small caudal fin supported by possibly cartilaginous rods (Fig. 2i)[46,47]. Superficially, this agrees with vertebrate morphology, although the absence of more typical vertebrate structures, such as gill arches, remains puzzling. The most contentious question was whether euconodont denticle tissues were homologous with vertebrate teeth and odontodes (skin denticles; Fig. 3a), a scenario that was advocated by some[48], but rejected by others[49]. This controversy was finally resolved with the demonstration, by means of high-resolution microtomographic techniques, that euconodont denticle structure and growth were largely at odds with that of vertebrate odontodes[43]. Nevertheless, there remains a chordate- or vertebrate-like aspect to the euconodont body imprints, which does not preclude their position as either stem vertebrates or stem cyclostomes (Fig. 4). During the past 15 years, euconodonts were almost constantly considered in phylogenetic analyses of early vertebrates, and their position as basal-most stem gnathostomes was essentially supported by the presence of the phosphatic denticles[50], which were then assumed to be homologues of gnathostome hard tissues, but lacking in all cyclostomes. However, an old hypothesis that euconodonts might be allied to cyclostomes, and more specifically hagfishes, periodically reappears in the literature[51–53]. For example, the enigmatic Carboniferous protoconodont-like soft-bodied fossil *Conopiscius*[54] shows, like euconodont-bearing animals, a series of chevron-shaped myomeres, but a single pair of hollow, weakly mineralized denticles[52]. It has been suggested that conodont denticles were partly or entirely capped with a keratinous tissue[51,52], which would remain in living cyclostomes. This hypothesis has now been dismissed[55]. The controversy about the homology of the para- and euconodont elements now seems to be settled, and all that soft-tissue data can currently suggest is that euconodonts might be either stem vertebrates, stem cyclostomes (Fig. 4) or, less likely, stem lampreys or stem hagfishes.

Other possible soft-bodied fossil chordates occur here and there, notably in Silurian to Carboniferous rocks, and some are more readily recognized as vertebrates, because they superficially resemble living hagfishes or lampreys. However, the risk of being misled by wishful thinking when making such comparisons is much the same as with odd Cambrian fossils. The fossil lampreys came as a surprise when first discovered in Carboniferous 300-Myr-old rocks, because of their striking overall resemblance to modern forms. *Mayomyzon*[15] (Fig. 2j), preserved as an imprint from the Mazon Creek Lagerstätte in Illinois, looks somewhat like a radiograph of a small modern lamprey. The image shows the outline of the body, the gill pouches and the characteristic cartilages of the 'tongue' apparatus. Other fossil lampreys turned up in the Carboniferous[16] and the Late Devonian (around 360 Ma)[17]. The latter, *Priscomyzon* (Fig. 2k), shows annular cartilage that supports the characteristic oral funnel. The two presumed fossil hagfishes, both coeval with *Mayomyzon*, are more questionable. *Myxinikela*[19] (Fig. 2l) has cartilage imprints and tentacles that do resemble those of hagfishes, but *Myxineidus*[20] was referred to as a hagfish based only on the impression of two V-shaped rows of keratinous teeth that resemble those of living hagfishes. The Mazon Creek Lagerstätte has also yielded peculiar presumed soft-bodied jawless vertebrates, *Pipiscius* and *Gilpichthys*[56]. The former has a lamprey-like oral funnel, and the latter shows possible impressions of sharp, non-mineralized teeth that resemble those of hagfishes[57]. Yet this interpretation remains controversial[58].

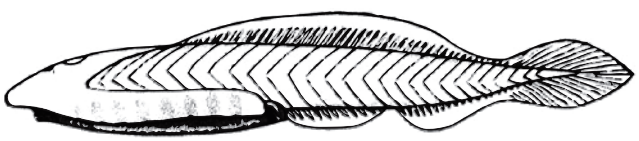
Another peculiar Palaeozoic soft-bodied vertebrate is *Jamoytius* (Fig. 2m)[59], from the Silurian (about 438 Ma) of Scotland, which was first regarded as an 'ancestral chordate'. New investigations show that the series of W-shaped imprints on the trunk of *Jamoytius* are not merely soft-tissue imprints of myomeres, but weakly mineralized scales[60]. With its median nostril and about ten gill openings, *Jamoytius* is otherwise suggestive of a lamprey and is often regarded as closely related to the younger, Devonian euphaneropids (*Euphanerops*, *Cornovichthys*, *Achanarella*; Fig. 2n), whose morphology is now best known from well-preserved 380-Myr-old *Euphanerops* material from the Late Devonian Miguasha Lagerstätte in Canada. Young individuals of *Euphanerops* are preserved as soft-tissue stains, but large individuals also show peculiar spongy calcifications of various elements of the endoskeleton (the internal, cartilaginous or bony skeleton of vertebrates), notably the fin radials, gill bars, vertebral elements, and elements that resemble the 'tongue' and annular cartilages of lampreys[61]. The most peculiar feature of *Euphanerops* is the large number (about 30 pairs) of gill bars that form its lamprey-like gill basket and extend back to the anal region. This is confirmed by a three dimensionally preserved specimen that shows impressions of the gill filaments[62]. Besides this feature, the overall appearance of *Euphanerops* resembles that of an anaspid, a group of Silurian–Devonian 'ostracoderms' that were long thought to be ancestral to lampreys, but are now regarded as being among the basal-most stem gnathostomes[60,61,63] (Fig. 4). Like anaspids, *Euphanerops* displays a long, posteroventrally slanting tail and a large anal fin, suggested to be paired — a unique case among vertebrates[64]. However, this requires confirmation, as does the elongate, paired ventrolateral fins that seem to have extended ventrally to the gill basket[61]. Whatever their relationships to *Jamoytius*, euphaneropids did not possess mineralized scales, but do have some endoskeletal characters uniquely shared with lampreys[65].

Finally, *Palaeospondylus*, from the Middle Devonian (390 Ma) of Scotland is still the most enigmatic early vertebrate, although it is known by hundreds of specimens. It is not preserved as a mere imprint, but clearly displays a vertebral column, a caudal fin with radials and fin supports, possible paired appendages, and its skull consists of several peculiar skeletal elements that cannot be clearly homologized with classic components of the vertebrate skull, be it a cyclostome or a gnathostome[66,67]. All of its skeletal elements are exclusively made up of a spongy calcified matter, which resembles that of the calcified endoskeleton of *Euphanerops*[61], and

BOX 1

Fossils and 'ancestors'

When the first description of the myllokunmingiids was published[39], early vertebrate palaeontologists were struck by the resemblance between these Lower Cambrian soft-bodied fossils from Chengjiang and various imaginary reconstructions of an ancestral vertebrate published during the twentieth century. For example, myllokunmingiids surprisingly resemble this imaginary reconstruction of an 'ancestral cephalochordate' (amphioxus) (see Figure) published at a time when some zoologists considered the absence of a complex head in living cephalochordates could be secondary. This reconstruction is a curious mix of a rather vertebrate-like, and even a 'ostracoderm'-like head, and some cephalochordate characters. It was thus intended to suggest that the overall morphology of the common ancestor to cephalochordates and vertebrates was rather vertebrate-like. Do such reconstructions of an entirely hypothetical 'ancestor', essentially based on inferences from extant and some fossil vertebrates, influence the way we interpret odd and poorly preserved soft-bodied fossils? Or do such fossils lead us to search for such old and supposedly prophetic reconstructions to justify intuitions? Although palaeontologists try to take a cold look at characters, it is probable that such reconstructions, based on the tree of life in vogue at a given time, unconsciously affect the way researchers look at certain fossils and favour wishful thinking when in search of ancestors. This was probably also the case for the interpretation of *Pikaia*. Image adapted with permission from ref. 42.



is therefore interpreted as calcified cartilage. Its resemblance to embryonic cartilage of extant osteichthyans (bony jawed vertebrates) has even led to the suggestion that *Palaeospondylus* might be a peculiar bony fish that failed to develop bone[68]. The anatomy of *Palaeospondylus* has been described, and this 'fish' has been tentatively referred to as practically all major fossil and extant vertebrate groups: hagfishes, lampreys, 'placoderms' (extinct armoured jawed fish), chondrichthyans (sharks, rays and chimaeras), teleosts, lungfish larvae and amphibian tadpoles[67,68]. All these interpretations are either dismissed or still debated. However, data on hagfish skeletal development[69] seem to enhance the superficial resemblance, already alluded to by some early authors, between the arrangement of certain elements of the *Palaeospondylus* skull and that of the cranial cartilages of late hagfish embryos. In addition, developmental data suggest that the absence of vertebral elements in hagfishes is probably secondary[70], and the vertebral column of *Palaeospondylus* may thus not preclude close relationships to hagfishes. Yet, no unambiguous character seems to be uniquely shared by hagfishes and *Palaeospondylus*.

These presumed soft-bodied chordates and vertebrates that were mostly devoid of hard tissue, except for occasional calcified cartilage, are generally collapsed and preserved as traces of variously transformed soft tissue[3,6]. Their reconstruction in 3D is often difficult, even by means of sophisticated techniques[60], and their descriptions are characteristically cautious. Should we simply forget about them? Do they provide us with any useful information? Or are they merely material support to our imagination, which is in turn guided by current views about the interrelationships of living animal groups? The art of reconstruction for palaeontologists is usually to put flesh on bones, but it is difficult when there is only decayed flesh and no bone! However, it is worth trying.

Hard-tissue data

Early vertebrate hard tissues are reputedly easier to identify. Their structure can be studied in detail by means of material or virtual (microtomographic) sections, eventually in 3D[71], and classic scanning electron microscopy techniques. Their characteristics can then be compared with those of living or more recent and well-known species. Nevertheless, palaeontologists are confronted with many of the same problems as for soft-tissue preservations when dealing with the earliest presumed vertebrate skeletal remains. The first clues to vertebrate hard tissues are that they are made of bioapatite; the tissues often show an ornamentation of tubercles (odontodes), or ridges, with a structure that resembles that of our teeth; they have dentine that contains thin canals for cell processes; eventually enamel (enameloid) is present; and there is a pulp cavity (Fig. 3a). Other useful characters may be the surface ultra-sculpture, the small spaces that housed bone cells, and the grooves or canals that housed lateral-line sense organs. The exoskeleton of the earliest, articulated and duly recognized vertebrates, such as arandaspids or astraspids (Fig. 3b, c), show at least some of these characters[13,72,73]. However, younger vertebrates known from complete specimens, such as the Silurian and Devonian anaspids or galeaspids[73,74], lack dentine, and many of the Cambrian to Silurian 'microremains', referred to as vertebrates owing to the aspect of their ornamentation or their scale-like shape, lack some of these characteristic tissues. Instead, they show other hard tissues that no longer exist, such as lamelline (acellular dentine)[8,73]. Therefore, the earliest evidence for possible vertebrate hard-tissue remains are barely less puzzling than the Cambrian soft-bodied animals.

The first controversy about these problematic skeletal fragments arose with the discovery of *Anatolepis* from the Lower Ordovician and Upper Cambrian[75,76]. *Anatolepis* is represented by minute phosphatic fragments ornamented with elongate tubercles (Fig. 3d), which vaguely resemble the exoskeletal ornamentation of certain Silurian–Devonian ostracoderms, notably heterostracans (Fig. 4). Therefore, *Anatolepis* was first regarded as a possible heterostracan; this was immediately contested by some, whereas others considered it plausible. At around the same time, *Anatolepis* was tentatively referred to an arthropod, but again this raised debate. Later studies of the tissue structure of these fragments using new techniques showed that the tubercles of *Anatolepis* were in fact hollowed by a pulp cavity capped by a somewhat dentine-like tissue, and connected by a lamellar tissue, which was perforated by thin vertical canals (Fig. 3e)[77]. Nevertheless, these new data failed to convince the sceptics[78]. *Anatolepis* may remain an enigma — as long as no articulated individual turns up. Other alleged Late Cambrian vertebrate bone fragments have been described from Australia[79] and superficially resemble the exoskeletal bone ornamentation of the Ordovician arandaspid *Porophoraspis*[11]; however, they are also strikingly similar to some Palaeozoic arthropod carapaces[78]. In sum, apart from the euconodonts, whose possible vertebrate affinities essentially rest on soft-tissue characters, there is no undisputed evidence for Cambrian vertebrates that possess a mineralized skeleton. By contrast, the following Ordovician period not only yields articulated vertebrates covered with extensive mineralized armour and scales, but also numerous isolated bone fragments and scales[80]. Most of these microremains, such as

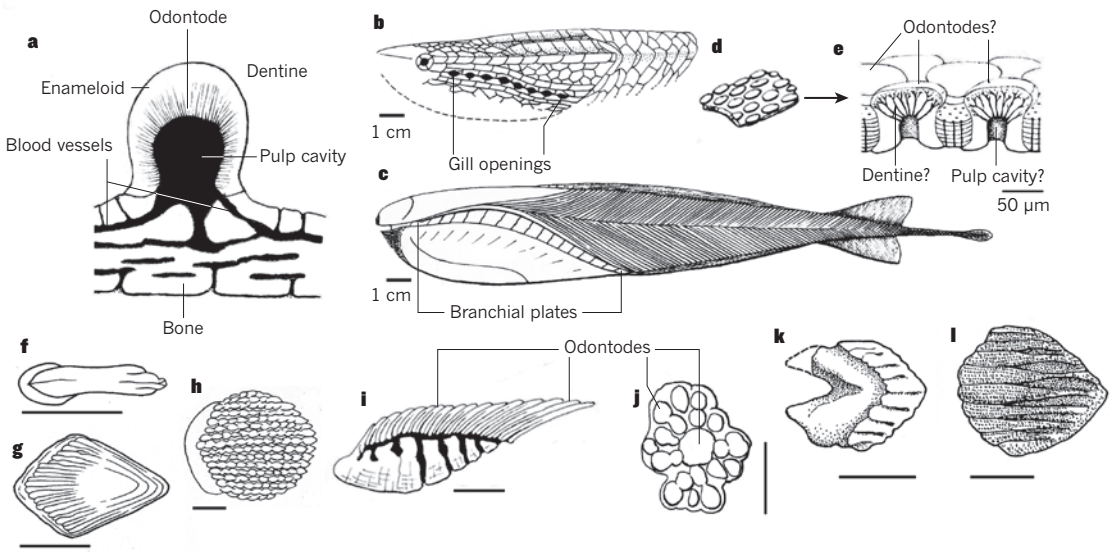


Figure 3 | Late Cambrian, Ordovician and early Silurian vertebrate exoskeletons. **a**, Ideal vertical section through a typical, ornamented vertebrate exoskeleton showing a tubercle (odontode) attached to a bony base (not to scale). **b**, **c**, The most complete articulated Ordovician vertebrates, *Astraspis* (**b**) and *Sacabambaspis* (**c**) (adapted from refs 86, 93). **d**, **e**, Exoskeleton fragment of the debated vertebrate *Anatolepis* (**d**) and vertical section of the possible odontodes (**e**) (adapted from ref. 77). **f**–**l**, Major types of isolated vertebrate scales retrieved from Upper Ordovician and Lower Silurian rocks: a thelodont (**f**), an 'acanthodian'(**g**), the possible chondrichthyan *Mongolepis* as an external view (**h**) and vertical section (**i**), and the vertebrates of uncertain affinities *Tesakoviaspis* (**j**), *Apedolepis* (**k**) and *Areyongalepis* (**l**). (**f**–**j** adapted from ref. 8 and **k**, **l** from ref. 82) Scale bars are 1 cm (**b**, **c**), 0.5 mm (**f**–**l**) and 50 µm (**e**).

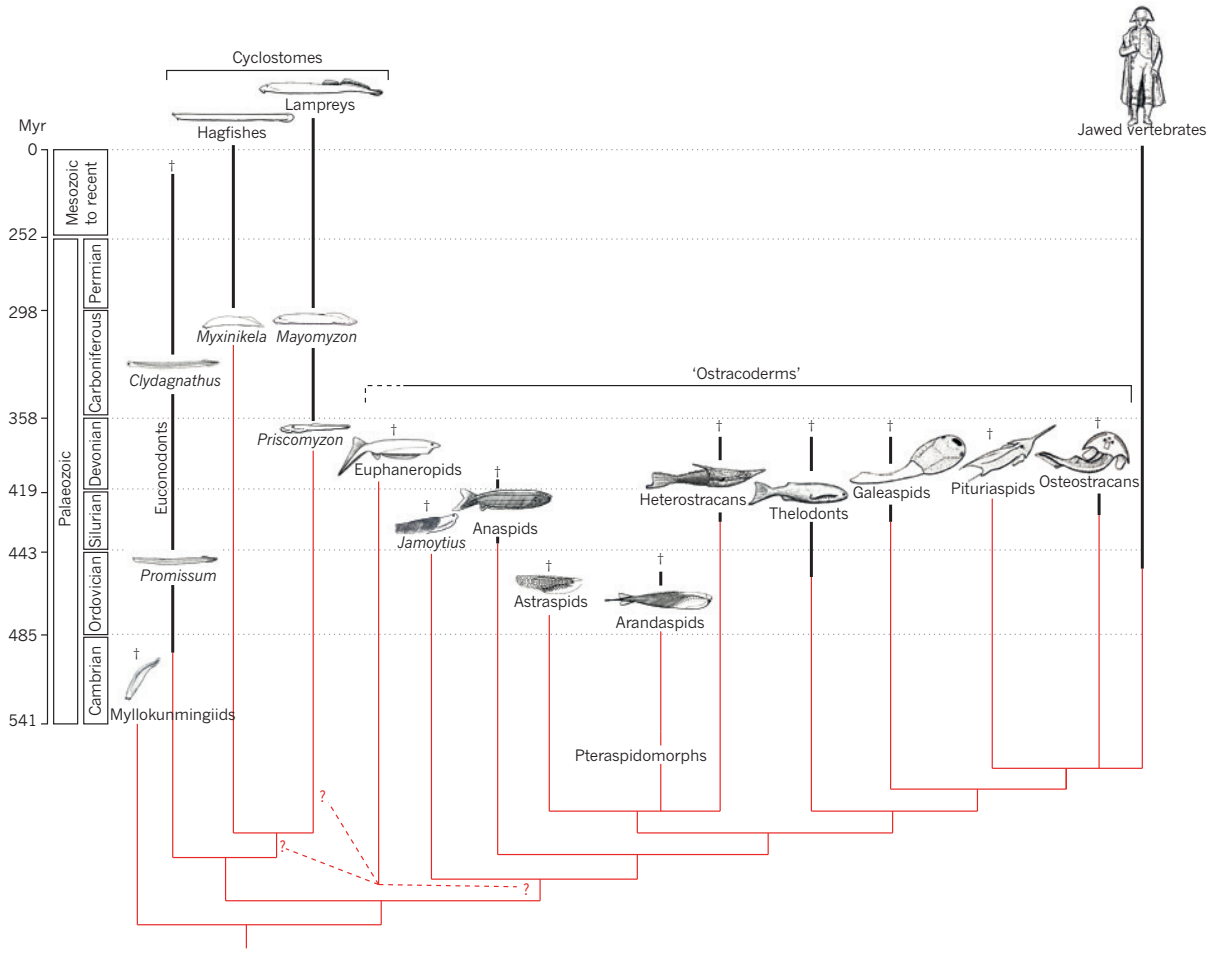


Figure 4 | Distribution through geological time (black bars), and patterns of interrelationships (red) of the major Palaeozoic jawless vertebrate groups and their extant relatives. Pattern of relationships adapted from ref. 60, except for the position of the euphaneropids. *Promissum* and *Clydagnathus* adapted from ref. 47; lampreys, hagfishes, *Myxinikela*, *Mayomyzon*, *Priscomyzon*, Euphaneropids, Anaspids, Heterostracans and Thelodonts adapted from ref. 65; *Jamoytius* adapted from ref. 60; *Astraspids* adapted from ref. 86; Arandaspids adapted from ref. 93. Not to scale. †Extinct groups.

Skiichthys[81] (a possible 'placoderm') or other scale-like elements, show at least some hard-tissue characters that are shared with younger vertebrate groups. However, others, such as *Areyongalepis* (Fig. 3k) and *Apedolepis* (Fig. 3l) are very puzzling[82]. Isolated vertebrate remains occur sporadically throughout most of the Ordovician and early Silurian and, despite their amazing diversity of hard-tissue structures, show an increasingly close resemblance to structures and ornamentations of the late Silurian and Devonian vertebrate groups, which are known from complete skeletons.

The three articulated Ordovician vertebrates, *Astraspis* (Fig. 3b), *Arandaspis* and *Sacabambaspis* (Fig. 3c)[11–13], and the bone assemblages of *Eriptychius*[83] and *Ritchieichthys*[84] show the overall morphology of the earliest vertebrates that have an extensive exoskeleton with a large head shield composed of either large plates or polygonal platelets, a posteriorly slanting series of numerous gill openings, and a scale-covered body and tail[85,86]. However, they provide no information about internal anatomy, apart from uninformative fragments of calcified cartilage in *Eriptychius*[83], and faint internal impressions of the gill pouches in *Astraspis* and *Sacabambaspis*. Orbits indicate the presence of eyes, and paired dorsal openings in arandaspids are interpreted as pineal foramina, but the position of nasal openings is unclear[57]. The lower lip of arandaspids is covered with a series of minute platelets, suggesting a filtering function, as in the younger heterostracans[87]. These articulated fossils may give the impression that all Ordovician fishes looked like big armoured tadpoles. However, the diversity of the scales and other microremains retrieved from coeval Ordovician rocks suggests that different morphologies may have existed already. *Porophoraspis* is regarded as an arandaspid, but some relatively large plates referred to as this genus are difficult to reconcile with the head-skeleton morphology of either *Arandaspis* or *Sacabambaspis*[11]. Among the isolated scales retrieved from Ordovician and Early Silurian rocks, some clearly belong to thelodonts (a group of 'ostracoderms'; Figs 3f, 4) and 'acanthodians' (Fig. 3g; presumed stem chondrichthyans). Both of these were known later by complete specimens, whereas others, such as *Mongolepis* (Fig. 3h, i), *Teslepis*, *Sodolepis* and *Tesakoviaspis* (Fig. 3j)[8], all presumed chondrichthyans (shark relatives), and still-unnamed forms[80] may have belonged to vertebrates that had an entirely micromeric (composed of minute scales) exoskeleton like that of sharks. Their body structure will remain unknown unless articulated material is discovered in some still-elusive Lagerstätte. Although some of these scales are, by default, referred to as chondrichthyans, they are in fact vertebrates in limbo.

After the Middle Ordovician, no articulated vertebrate turns up until the mid-Silurian (around 433 Ma), apart from the Late Ordovician euconodont *Promissum*[45]. Then, relatively complete representatives of the six major Silurian–Devonian 'ostracoderm' groups (anaspids, heterostracans, thelodonts, galeaspids, pituriaspids and osteostracans; Fig. 4) occur, and, shortly after (about 430 Ma) the earliest complete jawed vertebrates, notably 'placoderms'[10], 'acanthodians' and osteichthyans (bony fishes)[9]. Such articulated or well-preserved material is generally the key to suggesting a systematic position for some of the microremains from the Ordovician and early Silurian, and tracing back the distribution of these major groups through time (Fig. 4). Moreover, the number of anatomical characters that this material now offers us allows for better supported reconstructions of the interrelationships of these groups.

The phylogenetic trees of fossil and living vertebrates generally agree on the position of the 'ostracoderms' as a series of jawless stem gnathostomes,

with galeaspids, osteostracans (and possibly pituriaspids) as successive sister groups of the jawed vertebrates[50,57,60,88] (Fig. 4). This is partly because galeaspids and osteostracans have an extensively calcified or ossified endoskeleton, which preserves the cavities and canals that housed the brain, sensory capsules, nerves and blood vessels, including the pectoral girdles and fins in osteostracans, thereby providing a wealth of anatomical characters that can be compared with their homologues in jawed vertebrates[57,88,89]. However, the relationships of other 'ostracoderm' groups is poorly supported because they are devoid of a calcified endoskeleton, and their exoskeleton, which is sometimes entirely micromeric, provides indirect information about their internal anatomy in the form of faint impressions of, for example, gill pouches, brain, olfactory organs or labyrinth[57]. As is the case for heterostracans, but there are no data for anaspids, and only a few thelodonts provide some information[57,90,91]. Heterostracans are characterized by a single pair of common branchial openings, and are gathered with astraspids and arandaspids in the pteraspidomorphs (Fig. 4)[57,72]. However, apart from the presence of large median dorsal and ventral head plates made of acellular bone, and a similar honeycomb-like layer in the exoskeleton of heterostracans and arandaspids, shared derived characters that are unique to these three groups are scarce.

For almost a century, most debates about the relationships of the various 'ostracoderm' groups have been centred on the structure of the rostral part of the head: the olfactory organs, their relation to the hypophysis (pituitary) and the oral region. Classically, the dorsal position of the common nasal and hypophyseal duct of osteostracans and anaspids was compared with the condition in lampreys[92,93]. However, the recent description of the same region of the head in galeaspids has provided new insights[94]. The still elusive heterostracan and thelodont internal anatomy could possibly be reconstructed on the basis of that of galeaspids, with paired nasal sacs and an anteriorly directed hypophyseal duct. This would mean that a galeaspid-like anatomy might have been widespread among stem gnathostomes, and that the allegedly lamprey-like nasohypophyseal complex of osteostracans is independently derived from such a condition.

Fossils, phylogeny and technologies

It is sometimes said that fossils never, or rarely, overturn patterns of relationships based on extant organisms. Patterson[95] mentioned a few possible exceptions, notably the 'calcichordate theory'[96], which assumed that an ensemble of Palaeozoic echinoderm-like groups classically referred to as stylophorans are a paraphyletic array of stem chordates, stem cephalochordates, stem tunicates and stem vertebrates, the calcitic skeleton of which has been lost several times. It also suggested that tunicates, and not cephalochordates, were the closest extant relatives of vertebrates (contra to the then accepted relationships). This theory has raised heated controversies[97], but all stylophorans are now regarded as stem echinoderms. However, recent molecular phylogenies strongly support this tunicate–vertebrate relationship[98]. Tunicates and vertebrates are therefore gathered in a group called Olfactores, a name that, paradoxically, was erected in the framework of the calcichordate theory[96], because some stylophorans that were thought to be stem tunicates display internal structures that resemble vertebrate olfactory organs. Patterson[95] predicted that molecular sequence data would be the best test of the 'calcichordate theory', and, coincidently, the test seems to have been positive regarding tunicate relationships.

Regarding vertebrates, the hypothesis of living cyclostome paraphyly (that lampreys are more closely related to gnathostomes than to hagfishes) was only based on phenotypic data derived from extant species[99]. Palaeontological data have been merely adapted to this pattern of relationships, because of the long-lasting conviction that certain 'ostracoderms' (osteostracans and anaspids) were most closely related to lampreys[57,93]. More accurate character analyses later showed that 'ostracoderms' were exclusively stem gnathostomes, and the recent revival of cyclostome monophyly had no major bearing on their interrelationships[60]. None of the fossils discussed earlier, be they soft-body imprints, bone fragments, scales or articulated skeletons, seems currently liable to overturn the interrelationships of the major extant vertebrate groups. However, they provide a minimal age for certain characters (thus the groups they define), and may reveal unsuspected character combinations that allow the reconstruction of the stepwise assembly of novel body plans that foreshadow major evolutionary transitions. This is, for example, what 'ostracoderms' document with the succession of characters that make up the jawed vertebrate body: the rise of the exoskeleton, cellular bone, endoskeletal bone, enlarged cerebellum or pectoral fins, but they are still rather powerless in providing a scenario for the rise of jaws, which is largely left in the hands of evolutionary developmental biologists. Nevertheless, the recent consideration of braincase anatomy in the basal-most 'placoderms' suggests that the anatomical gap between such 'ostracoderms' as galeaspids and osteostracans, and the earliest jawed vertebrates, may not have been that large, and that the prerequisites to the rise of jaws were already there[100].

The future of early vertebrate palaeontology rests on the quality of the data it can provide, especially on fossils derived from crucial periods, such as the Late Cambrian, Early Ordovician and early Silurian. Early vertebrates are generally difficult material, compressed or crushed in hard rocks. Throughout the twentieth century, some early vertebrate palaeontologists gave much weight to the then new preparation techniques[57]. Nowadays, they would be amazed by the quality of the data obtained from high-resolution X-ray microtomography. Also, soft tissues preserved as mere stains can be studied by element mapping that provides information on the fossilization process and sometimes the nature of the preserved tissues themselves. Armed with these non-destructive techniques, early vertebrate palaeontologists can considerably refine their observations and must not be afraid of proposing audacious interpretations of these miserable remains, even though 'squashed slugs' may be slippery! ■

Received 9 September 2014; accepted 20 March 2015.

1. Hou, X.-G. *et al. The Cambrian Fossils of Chengjiang, China: the Flowering of Early Animal Life* (Blackwell Publishing, 2007).
2. Royal Ontario Museum. *The Burgess Shale* http://www.burgess-shale.rom.on.ca (Royal Ontario Museum, 2011).
3. Briggs, D. E. G. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annu. Rev. Earth Planet. Sci.* **31,** 275–301 (2003).
4. Sansom, R. S., Gabbott, S. E. & Purnell, M. A. Decay of chordate characters causes bias in fossil interpretation. *Nature* **463,** 797–800 (2010).
5. Sansom, R. S. Gabbott, S. E. & Purnell M. A. Decay of vertebrate characters in hagfish and lamprey (Cyclostomata) and the implications for the vertebrate fossil record. *Proc. R. Soc. Lond. B* **278,** 1150–1157 (2011).
6. Sansom, R. S., Gabbott, S. E. & Purnell, M. A. Atlas of vertebrate decay: a visual and taphonomic guide to fossil interpretation. *Palaeontology* **56,** 457–474 (2013).
7. Turner, S. in *Advances in the Origin and Early Radiation of Vertebrates* (eds Arratia, G., Wilson, M.V.H. & Cloutier, R.) 67–94 (Pfeil, 2004).
8. Karatayute-Talimaa, V. N. Determination methods for the exoskeletal remains of early vertebrates. *Fossil Record* **1,** 21–51 (1998).
   An important review of the tissue structure in Early Palaeozoic microremains.
9. Zhu, M., Zhao, W., Jia, L., Qiao, T. & Qu, Q. The oldest articulated osteichthyan reveals mosaic gnathostome characters. *Nature* **458,** 469–474 (2009).
10. Zhu, M. *et al*. A Silurian placoderm with osteichthyan-like marginal jaw bones. *Nature* **502,** 188–193 (2013).
11. Ritchie, A. & Gilbert-Tomlinson, J. First Ordovician vertebrates from the southern hemisphere. *Alcheringa* **1,** 351–368 (1977).
12. Gagnier, P.-Y. *Sacabambaspis janvieri*, vertébré ordovicien de Bolivie. 1, analyse morphologique [in French]. *Ann. Paleontol.* **79,** 19–69 (1993).
13. Sansom, I. J., Smith, M. P., Smith, M. M. & Turner, P. *Astraspis* — the anatomy and histology of an Ordovician fish. *Palaeontology* **40,** 625–643 (1997).
14. Donoghue, P. C. J. & Purnell, M. A. Genome duplication, extinction and vertebrate evolution. *Trends Ecol. Evol.* **20,** 312–319 (2005).
15. Bardack, D. & Zangerl, R. First fossil lamprey: a record from the Pennsylvanian of Illinois. *Science* **162,** 1265–1267 (1968).
16. Janvier, P. & Lund, R. *Hardistiella montanensis* N. gen. et sp. (Petromyzontida) from the Lower Carboniferous of Montana, with remarks on the affinities of lampreys. *J. Vert. Paleontol.* **2,** 407–413 (1983).
17. Gess, R. W., Coates, M. I. & Rubidge, B. S. A lamprey from the Devonian of South Africa. *Nature* **443,** 981–984 (2006).
18. Chang, M. M., Zhang, J. & Miao, D. A lamprey from the Cretaceous Jehol biota of China. *Nature* **441,** 972–974 (2006).
19. Bardack, D. First fossil hagfish (Myxinoidea): a record from the Pennsylvanian of Illinois. *Science* **254,** 701–703 (1991).
20. Poplin, C., Sotty, D. & Janvier, P. Un Myxinoïde (Craniata, Hyperotreti) dans le *Konservat-Lagerstätte* Carbonifère supérieur de Montceau-les-Mines (Allier, France) [in French]. *CR. Acad. Sci. II A* **332,** 345–350 (2001).
21. Graham, A., Butts, T., Lumsden, A. & Kiecker, C. What can vertebrates tell us about segmentation? *EvoDevo* **5,** 24 (2014).
22. Conway Morris, S. & Caron, J.-B. *Pikaia gracilens* Walcott, a stem-group chordate from the Middle Cambrian of British Columbia. *Biol. Rev. Camb. Philos. Soc.* **87,** 480–512 (2012).
23. Gould, S. J. *Wonderful Life: The Burgess Shale and the Nature of History* (W.W. Norton & Co, 1990).

24. Chen, J. Y., Dzik, J., Edgecombe, G. D., Ramskjöld, L. & Zhou, G. Q. A possible Early Cambrian chordate. *Nature* **377,** 720–722 (2002).
25. Shu, D. et al. A new species of Yunnanozoan with implications for deuterostome evolution. *Science* **299,** 1380–1384 (2003).
26. Chen, J.-Y., Huang, D.-Y. & Li, C.-W. An early Cambrian craniate-like chordate. *Nature* **402,** 518–522 (1999).
27. Holland, N. D. & Chen, J. Origin and early evolution of the vertebrates: new insights from advances in molecular biology, anatomy, and palaeontology. *Bioessays* **23,** 142–151 (2001).
28. Mallatt, J. & Chen, J. Fossil sister group of craniates: predicted and found. *J. Morphol.* **258,** 1–31 (2003).
The most detailed description of Yunnanozoan anatomy.
29. Mallatt, J., Chen, J. & Holland, N. D. Comment on "A new species of ynnanozoan, with implications for deuterostome evolution". *Science* **300,** 1372 (2003).
30. Shu, D. & Conway Morris, S. Response to Comment on "A new species of yunnanozoan with implications for deuterostome evolution". *Science* **300,** 1372 (2003).
31. Shu, D.-G. *et al*. Primitive deuterostomes from the Chengjiang Lagerstätte (Lower Cambrian, China). *Nature* **414,** 419–424 (2001).
32. Gee, H. On being vetulicolian. *Nature* **414,** 407–409 (2001).
33. Caron, J.-B. *Banffia constricta*, a putative vetulicolid from the Middle Cambrian Burgess Shale. *Trans. R. Soc. Edinb. Earth Sci.* **96,** 95–111 (2005).
34. Shu, D.-G., Conway Morris, S. & Zhang, X.-L. A *Pikaia*-like chordate from the Lower Cambrian of China. *Nature* **384,** 157–158 (1996).
35. Shu, D.-G., Chen, L., Han, J. & Zhang, X. L. An Early Cambian tunicate from China. *Nature* **411,** 472–473 (2001).
36. Chen, J. Y. *et al*. The first tunicate from the early Cambrian of South China. *Proc. Natl Acad. Sci. USA* **100,** 8314–8318 (2003).
37. Ritchie, A. *Ainiktozoon loganense* Scourfield, a protochordate? From the Silurian of Scotland. *Alcheringa* **9,** 117–142 (1985).
38. Van der Brugghen, W., Schram, F. R. & Martill, D. M. The fossil *Ainiktozoon* is an Arthropod. *Nature* **385,** 589–590 (1997).
39. Shu, D.-G. *et al*. Lower Cambrian vertebrates from South China. *Nature* **402,** 42–46 (1999).
40. Shu, D. G. *et al*. Head and backbone of the Early Cambrian vertebrate *Haikouichthys*. *Nature* **421,** 526–529 (2003).
41. Conway Morris, S. & Caron, J.-B. A primitive fish from the Cambrian of North America. *Nature* **512,** 419–422 (2014).
42. Holmgren, N. & Stensiö, E. in *Handbuch des Vergleichenden Anatomie der Wirbeltiere* [in German] Vol. 4 (eds Bolk, L., Göppert, E., Kallius, E. & Lubosch, W.) 233–500 (Urban & Schwarzenberg, 1936).
43. Murdock, D. J. E. *et al*. The origin of conodonts and of vertebrate mineralized skeletons. *Nature* **502,** 546–549 (2013).
44. Briggs, D. E. G., Clarkson, E. N. K. & Aldridge, R. J. The conodont animal. *Lethaia* **20,** 1–14 (1983).
45. Gabbott, S. E., Aldridge, R. J. & Theron, J. N. A giant conodont with preserved muscle tissue from the Upper Ordovician of South Africa. *Nature* **374,** 800–803 (1994).
46. Aldridge, R. J., Briggs, D. E. G., Smith, M. P., Clarkson, E. N. K. & Clark, D. N. L. The anatomy of conodonts. *Philos. Trans. R. Soc. Lond. B* **340,** 405–421 (1993).
47. Purnell, M. A. Large eyes and vision in conodonts. *Lethaia* **28,** 187–188 (1995).
48. Donoghue, P. C. J. Growth and patterning in the conodont skeleton. *Philos. Trans. R. Soc. Lond. B* **353,** 633–666 (1998).
49. Turner, S. *et al*. False teeth: conodont-vertebrate phylogenetic relationships revisited. *Geodiversitas* **32,** 545–594 (2010).
50. Donoghue, P. C. J., Forey, P. L. & Aldridge, R. J. Conodont affinity and chordate phylogeny. *Biol. Rev. Camb. Philos. Soc.* **75,** 191–251 (2000).
51. Kreijsa, R. J., Bringas, P. & Slavkin, H. A neontological interpretation of conodont elements based on agnathan cyclostome tooth structure, function and development. *Lethaia* **23,** 359–378 (1990).
52. Dzik, J. Conodont affinity of the enigmatic Carboniferous chordate *Conopiscius*. *Lethaia* **42,** 31–38 (2009).
53. Goudemand, N., Orchard, M. J., Urdy, S., Bucher, H. & Tafforeau, P. Synchrotron-aided reconstruction of the conodont feeding apparatus and implications for the mouth of the first vertebrates. *Proc. Natl Acad. Sci. USA* **108,** 8720–8724 (2011).
54. Briggs, D. E. G. & Clarkson, E. N. K. An enigmatic chordate from the Lower Carboniferous Granton "shrimp-bed" of the Edinburgh district, Scotland. *Lethaia* **20,** 107–115 (1987).
55. Aldridge, R. J. & Donoghue, P. C. J. in *The Biology of Hagfishes* (eds Jørgensen, J. M., Lomholt, J. P., Weber, R. E. & Malte, H.) 16–31 (Chapman and Hall, 1998).
56. Bardack, D. & Richardson, E. S. Jr. New agnathous fishes from the Pennsylvanian of Illinois. *Fieldiana: Geology* **33,** 489–510 (1977).
57. Janvier, P. *Early Vertebrates* (Oxford Univ. Press, 1996).
This text is a general overview of early vertebrate anatomy and relationships.
58. Bardack, D. in *The Biology of Hagfishes* (eds Jørgensen, J. M., Lomholt, J. P., Weber, R. E. & Malte, H.) 3–14 (Chapman and Hall, London, 1998).
59. White, E. I. *Jamoytius kerwoodi*, a new chordate from the Silurian of Lanarkshire. *Geol. Mag.* **83,** 89–97 (1946).
60. Sansom, R. S., Freedman, K., Gabbott, S. E., Aldridge, R. J. & Purnell, M. A. Taphonomy and affinity of an enigmatic Silurian vertebrate, *Jamoytius kerwoodi* White. *Palaeontology* **53,** 1393–1409 (2010).
61. Janvier, P. & Arsenault, M. The anatomy of *Euphanerops longaevus* Woodward, 1900, an anaspid-like jawless vertebrate from the Upper Devonian of Miguasha, Quebec, Canada. *Geodiversitas* **29,** 143–216 (2007).
62. Janvier, P., Desbiens, S. & Willett, J. A. & Arsenault, M. Lamprey-like gills in a gnathostome-related Devonian jawless vertebrate. *Nature* **440,** 1183–1185 (2006).
63. Blom, H. New birkeniid anaspid from the Lower Devonian of Scotland and its phylogenetic implications. *Palaeontology* **55,** 641–652 (2012).
64. Sansom, R. S., Gabbott, S. E. & Purnell, M. A. Unusual anal fin in a Devonian jawless vertebrate reveals complex origins of paired appendages. *Biol. Lett.* **9,** 20130002 (2013).
65. Janvier, P. Modern look for ancient lamprey. *Nature* **443,** 921–924 (2006).
66. Sollas, W. J. & Sollas, I. B. An account of the Devonian fish, *Palaeospondylus gunni*, Traquair. *Phil. Trans. R. Soc. Lond. B* **196,** 267–294 (1904).
67. Moy Thomas, J. A. The Devonian fish *Palaeospondylus gunni* Traquair. *Philos. Trans. R. Soc. Lond. B* **230,** 391–413 (1940).
68. Johanson, Z., Kearsley, A., Den Blaauwen, J., Newman, M. & Smith, M. M. No bone about it: an enigmatic Devonian fossil reveals a new skeletal framework – a potential loss of gene regulation. *Semin. Cell Dev. Biol.* **21,** 414–423 (2010).
69. Oisi, Y., Ota, K. G., Fujimoto, S. & Kuratani, S. S. Development of the chondrocranium in hagfishes, with special reference to the early evolution of vertebrates. *Zoolog. Sci.* **30,** 944–961 (2013).
70. Janvier, P. All vertebrates do have vertebrae. *Curr. Biol.* **21,** R661–R663 (2011).
71. Sanchez, S., Ahlberg, P. E., Trinajstic, K. M., Mirone, A. & Tafforeau, P. Three-dimensional synchrotron virtual palaeohistology: A new insight into the world of fossil bone microstructure. *Microsc. Microanal.* **18,** 1095–1105 (2012).
72. Sansom, I. J., Donoghue, P. C. J. & Albanesi, G. Histology and affinity of the earliest armoured vertebrate. *Biol. Lett.* **1,** 446–449 (2005).
73. Sire, J.-Y., Donoghue, P. C. J. & Vikaryous, M. K. Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *J. Anat.* **214,** 409–440 (2009).
This is an updated review of exoskeletal hard tissues in early fishes.
74. Wang, N.-Z., Donoghue, P. C. J., Smith, M. M. & Sansom, I. J. Histology of the galeaspid dermoskeleton and endoskeleton, and the origin and early evolution of the vertebrate cranial endoskeleton. *J. Vert. Paleontol.* **25,** 745–756 (2005).
75. Bockelie, T. G. & Fortey, R. A. An early Ordovician vertebrate. *Nature* **260,** 36–38 (1976).
76. Repetski, J. E. A fish from the Upper Cambrian of North America. *Science* **200,** 529–531 (1978).
77. Smith, M. P., Sansom, I. J. & Repetski, J. E. Histology of the first fish. *Nature* **380,** 702–704 (1996).
78. Smith, M. P., Sansom, I. J. & Cochrane, K. D. in *Major Events in Early Vertebrate Evolution* (ed. Ahlberg, P. E.) 67–84 (Taylor and Francis, 2001).
79. Young, G. C., Karatayute-Talimaa, V. N. & Smith, M. M. A possible Late Cambrian vertebrate from Australia. *Nature* **383,** 810–812 (1996).
80. Sansom, I. J., Smith, M. M. & Smith, M. P. in *Major Events in Early Vertebrate Evolution* (ed. Ahlberg, P. E.) 156–171 (Taylor and Francis, 2001).
81. Smith, M. M. & Sansom, I. J. Exoskeletal micro-remains of an Ordovician fish from the Harding Sandstone of Colorado. *Palaeontology* **40,** 645–658 (1997).
82. Young, G. C. Ordovician microvertebrate remains from the Amadeus Basin, central Australia. *J. Vert. Paleontol.* **17,** 1–25 (1997).
83. Denison, R. H. Ordovician vertebrates from Western United States. *Fieldiana: Geology* **16,** 131–192 (1967).
84. Sansom, I. J., Haines, P. W., Andreev, P. & Nicoll, R. S. A new pteraspidomorph from the Nibil Formation (Katian, Late Ordovician) of the Canning Basin, Western Australia. *J. Vert. Paleontol.* **33,** 764–769 (2013).
85. Pradel, A., Sansom, I. J., Gagnier, P.-Y., Cespedes, R. & Janvier, P. The tail of the Ordovician fish Sacabambaspis. *Biol. Lett.* **3,** 72–75 (2007).
86. Janvier, P. in *Recent Advances in the Origin and Early Radiation of Vertebrates* (eds Arratia, G., Wilson, M. V. H. & Cloutier, R.) 29–52 (Pfeil, 2004).
87. Purnell, M. A. Feeding in extinct jawless heterostracan fishes and testing scenarios of early vertebrate evolution. *Proc. R. Soc.* **269,** 83–88 (2002).
88. Janvier, P. in *Major Events in Early Vertebrate Evolution* (ed. Ahlberg, P. E.) 172–186 (Taylor and Francis, 2001).
89. Janvier, P., Arsenault, M. & Desbiens, S. Calcified cartilage in the paired fins of the osteostracan *Escuminaspis laticeps* (Traquair 1880), from the Late Devonian of Miguasha (Québec, Canada), with a consideration of the early evolution of the pectoral fin endoskeleton in vertebrates. *J. Vert. Paleontol.* **24,** 773–779 (2004).
90. Donoghue, P. C. J. & Smith, M. P. The anatomy of *Turinia pagei* (Powrie) and the phylogenetic status of the Thelodonti. *Trans. R. Soc. Edinb. Earth Sci.* **92,** 15–37 (2001).
91. Märss, T. & Wilson, M. V. H. Buccopharyngo-branchial denticles of *Phlebolepis elegans* Pander (Thelodonti, Agnatha). *J. Vert. Paleontol.* **23,** 601–612 (2008).
92. Stensiö, E. A. The Devonian and Downtonian vertebrates of Spitsbergen. 1. Family Cephalaspidae. *Skr. Svalbard Ishav.* **12,** 1–391 (1927).
93. Janvier, P. Early jawless vertebrates and cyclostome origins. *Zoolog. Sci.* **25,** 1045–1056 (2008).
94. Gai, Z., Donoghue, P. C. J., Zhu, M., Janvier, P. & Stampanoni, M. Fossil jawless fish from China foreshadows early jawed vertebrate anatomy. *Nature* **476,** 324–327 (2011).
95. Patterson, C. Significance of fossils in determining evolutionary relationships. *Annu. Rev. Ecol. Syst.* **12,** 195–223 (1981).
96. Jefferies, R. P. S. *The Ancestry of the Vertebrates* (British Museum (Natural History), 1996).
This is an extensive account of the calcichordate theory.
97. Gee, H. *Before the Backbone: Views on the Origin of the Vertebrates* (Chapman & Hall, 1996).
This text is a review of fossil-based theories about the origin of vertebrates.
98. Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. Tunicate and not cephalochordate are the closest living relatives of vertebrates. *Nature* **439,** 965–968 (2006).
99. Løvtrup, S. *The Phylogeny of Vertebrata* (Wiley, 1977).
100. Dupret, V., Sanchez, S., Goujet, D., Tafforeau, P. & Ahlberg, P. E. A primitive placoderm sheds light on the origin of the jawed vertebrate face. *Nature* **507,** 500–503 (2014).

Acknowledgements I thank M. Friedman, M. Brazeau, P. Donoghue, R. Sansom and J. Keating for their helpful discussions. I also thank all the authors who allowed me to adapt their published figures.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The author declares no competing financial interests. Readers are welcome to comment on the online version of this paper at go.nature.com/ym67im. Correspondence should be addressed to P.J. (janvier@mnhn.fr).

REVIEW

doi:10.1038/nature14438

The origin and early phylogenetic history of jawed vertebrates

Martin D. Brazeau[1] & Matt Friedman[2]

Fossils of early gnathostomes (or jawed vertebrates) have been the focus of study for nearly two centuries. They yield key clues about the evolutionary assembly of the group's common body plan, as well the divergence of the two living gnathostome lineages: the cartilaginous and bony vertebrates. A series of remarkable new palaeontological discoveries, analytical advances and innovative reinterpretations of existing fossil archives have fundamentally altered a decades-old consensus on the relationships of extinct gnathostomes, delivering a new evolutionary framework for exploring major questions that remain unanswered, including the origin of jaws.

Jawed vertebrates (gnathostomes) comprise more than 99% of living vertebrate species, including humans. This diversity is built on features including jaws, teeth, paired appendages, and specialized embryonic and skeletal tissues (Box 1); centuries of research have attempted to explain their origins[1–7]. In particular, jaws and paired appendages have become flagship systems in the study of evolutionary novelty[5,7] — a key research programme in evolutionary biology[8].

The deepest split in the modern gnathostome tree is that between the chondrichthyans (sharks, rays and chimaeras) and the osteichthyans (bony fishes and tetrapods). This divergence occurred in the Palaeozoic era, at least 423 million years ago (Ma)[9], leaving a vast temporal and evolutionary gulf between modern lineages, with ample time for new innovations to overwrite primitive conditions. These complexities compel researchers to turn to the Palaeozoic fossil record to elucidate the origin of jawed vertebrates. A few well-preserved fossil taxa from a handful of Silurian–Permian sites in Europe and North America[10] shaped late nineteenth- and early twentieth-century hypotheses of gnathostome evolution[1,11,12] (Fig. 1). Many of these narratives persist to this day, either implicitly or explicitly. However, fossils once hailed as avatars for scenarios of jaw[12,13] or fin[1,14] origins often turn out to be specialized rather than primitive after phylogenetic investigation[15,16]. Until they are placed in a evolutionary tree, Palaeozoic fossils are mute on the question of gnathostome origins.

In this Review, we examine the progress made in the past two decades on the study of early gnathostome interrelationships, focusing on key fossil discoveries that have prompted a renewed intensity of phylogenetic investigation. Although tremendous advances have been made, much work remains before this research can deliver finely atomized transformational hypotheses such as those available for mammals[17], birds[18] and early tetrapods[19].

Phylogeny of extant gnathostomes

From the perspective of modern lineages alone, deep vertebrate phylogeny is well resolved and there is little disagreement about the branching patterns surrounding the gnathostome crown node (Box 1). Morphological[20] and molecular[21] data unambiguously indicate that chondrichthyans and osteichthyans are reciprocally monophyletic sister taxa. Together, they form a clade to the exclusion of the jawless cyclostomes: hagfishes and lampreys (Box 1). Molecular evidence strongly supports the monophyly of living agnathans with respect to jawed vertebrates. The long-standing morphological hypothesis indicated the union of lampreys and gnathostomes to the exclusion of hagfishes[10,22], but re-appraisal of traits in living species[23–25] and reconsideration of existing data sets[26] have exposed its weaknesses.

These established relationships put the study of early gnathostome evolution at an advantage. Modern taxa can be organized into a set of crown groups delimiting three stem lineages: the respective branches subtending Osteichthyes and Chondrichthyes, and the branch subtending their last common ancestor (Box 1). The palaeontological problem is reduced to phylogenetic placement of Palaeozoic fossils within this three-branch framework.

Palaeozoic jawed vertebrates and their phylogeny

In this section we outline the range of early gnathostome diversity and review the recent history of progress on their phylogenetic relationships.

Diversity of Palaeozoic jawed vertebrates

Putative examples of jawed vertebrates date to the Ordovician period[27–29], but the first definitive remains are of early Silurian age[30]. Early Devonian (419 Ma) mandibulate gnathostomes were already ecologically diverse[31] and, by the close of the Devonian (360 Ma), the first tetrapods and many of their adaptations for terrestriality had emerged[19].

Early jawed fishes are divided into four broad categories: ancient representatives of chondrichthyans and osteichthyans, along with two exclusively extinct assemblages: acanthodians and placoderms. The early chondrichthyan record is dominated by isolated denticles (scales), teeth and spines. The oldest records of scales attributed to chondrichthyans are from the earliest Silurian (around 443 Ma)[27], such as mongolepids[32]. Sinacanthids, represented by isolated spines that share histological similarities with chondrichthyans[33], are also known from the early Silurian (about 438 Ma)[30]. The oldest universally accepted chondrichthyans are substantially younger, represented by Early Devonian body fossils (around 400 Ma; Fig. 2e). Some of these specimens derive from the 'Malvinokaffric Realm', a cold-water Southern Hemisphere palaeobiogeographic province that yields distinctive jawed vertebrate faunas almost exclusively composed of acanthodians and chondrichthyans[34]. Articulated chondrichthyans remain rare throughout the Devonian, with most specimens known from the exceptional Late Devonian Cleveland Shale Lagerstätte (Fig. 1).

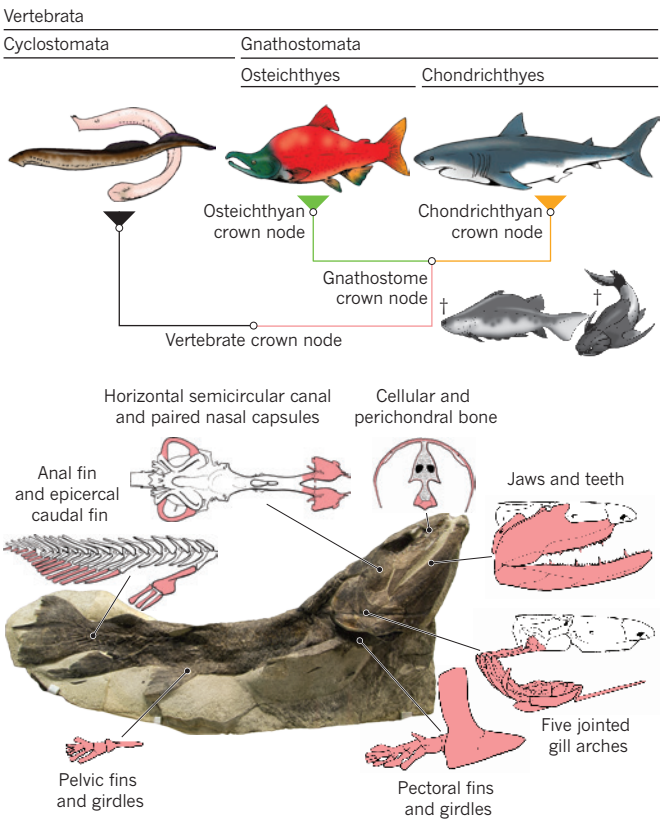
The late Silurian–Devonian osteichthyan record is considerably better than that of chondrichthyans owing to the armour of dermal plates and

[1]Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot SL5 7PY, UK. [2]Department of Earth Sciences, University of Oxford, South Parks Road, Oxford OX1 3AN, UK.

BOX 1

Crowns, stems and the characters of jawed vertebrates

Crown-, total- and stem-group concepts provide a useful framework for navigating evolutionary trees that include fossils. The tree shown in the figure reflects the most basic splits among living vertebrates. Crown groups comprise the last common ancestor of a group of living species plus all of its descendants, both fossil and modern. The gnathostome crown group includes the last common ancestor of osteichthyans (represented by a salmon) and chondrichthyans (represented by a shark) plus all of its descendants, and comprises all the green and orange parts of the tree. Total groups include the crown group of interest plus all extinct forms more closely related to that lineage than to any other living species. Here, the gnathostome total group is represented by all coloured parts of the tree. Stem groups are equal to a clade's total group minus its crown group, shown here by the pink lineage connecting the vertebrate and gnathostome crown nodes. Jawed vertebrates include all of the gnathostome crown, and the upper reaches of the gnathostome stem. The lower part of the gnathostome stem is populated by jawless ostracoderms, which are more closely related to jawed vertebrates than they are to modern jawless fishes. The principal task faced by palaeontologists is to fit fossil groups (such as acanthodians and placoderms; the dagger symbol indicates that they are extinct) within the genealogical framework for modern species. Monophyly of jawed vertebrates is evidenced by a series of shared morphological specializations including, but not limited to, jaws. Key gnathostome features are illustrated here for *Eusthenopteron* (Cleveland Museum of Natural History CMNH 8158, image courtesy of D. Chapman), an osteichthyan and relative of land vertebrates. These traits must have evolved along the gnathostome stem lineage, but without fossils it is impossible to determine the order in which — or when — they arose.



ossified endoskeleton typical of bony fishes. Consequently, osteichthyans have been intensively studied, with particular emphasis on sarcopterygians (lobe-finned fishes), reflecting their importance in reconstructing early stages of tetrapod evolution[19,35,36]. Lobe fins are known from the late Silurian (about 423 Ma)[9], but the earliest definitive remains of the other division of modern bony fish radiation — actinopterygians — are from the late Early or the earliest Middle Devonian, some 30 million years later[37]. Some scales and other skeletal detritus of late Silurian–Early Devonian age (about 427–400 Ma) are conventionally aligned with actinopterygians[38,39]. However, many — or perhaps all — of these taxa could represent stem osteichthyans[40,41] or even stem gnathostomes[42] (Fig. 3). As with chondrichthyans, early osteichthyans show some striking distributional patterns, including the conspicuous concentration of early members of major lobe-fin lineages in the latest Silurian and earliest Devonian of the South China Block[30] (Fig. 1). Outside of this restricted area, coeval bony fishes are limited to a handful of mostly fragmentary examples.

Several extinct groups join the familiar modern jawed vertebrate lineages. Armoured jawless fishes (ostracoderms) that are most often implicated as a jawed vertebrate sister group include: Middle Ordovician–Late Devonian (467–370 Ma) thelodonts, encompassing dorsoventrally flattened to cigar-shaped to deep-bodied forms[43] and bearing a shark-like shagreen of tiny scales; galeaspids, which are bottom-dwelling early Silurian–Late Devonian (439–370 Ma) fishes with flattened headshields that assume a bewildering variety of shapes and are found only in Chinese and Vietnamese deposits[44,45]; and osteostracans, which are another benthic group with spade-shaped headshields and are restricted to the middle Silurian–Late Devonian (433–372 Ma) of today's northern landmasses[45,46]. Two extinct jawed groups join this ostracoderm parade: placoderms, which are a species-rich and anatomically heterogeneous early Silurian–Late Devonian (435–360 Ma) assemblage characterized by heavy head and trunk armour and bony jaw plates[47]; and acanthodians, which are covered in tiny scales and bear well-developed spines along the leading edges of nearly all of their fins[10] that together inspire the moniker 'spiny sharks'. The earliest fossils associated with acanthodians are isolated scales from the latest Ordovician (around 444 Ma)[27]. More reliable remains are Silurian in age, with the group's record extending to early Permian deposits (about 295 Ma) that yield the best-known and last-surviving genus *Acanthodes*[48,49].

The evolution of gnathostome phylogeny

The current picture of Palaeozoic gnathostome relationships is the product of three phases of study. Throughout, researchers have benefitted from high-quality data, thanks to the early application of physical tomography by Stensiö and the 'Stockholm school'[50–52], followed by the maturation of acid-preparation techniques in the middle of the twentieth century[53–56] and the non-destructive computed tomography of the past 15 years[42,57–60].

The modern phase of research into gnathostome relationships began with the introduction of phylogenetic systematics to vertebrate palaeontology, which had previously focused on linking species from successive geological strata as an approximate ancestor–descendant chain. Monophyly of the major taxonomic divisions of early gnathostomes was assumed, and their relative relationships were largely inferred using evidence from European and North American fossils. Within a decade of the initial application of cladistics to early vertebrates, an imperfect consensus emerged that acanthodians were a clade of stem osteichthyans[48] and that placoderms were the immediate sister group of crown gnathostomes[61]. This framework would persist for more than 30 years[10], despite the intervening discovery and detailed description of fossils from Australia[53,56,62], China[30,63] and northern Canada[64] that provided fresh morphological information beyond the stagnating stable of classic Euramerican taxa.

The second phase began in the 1980s with a cladistic reinterpretation of the ostracoderms. Detailed anatomical reinvestigations of ostracoderm sublineages and numerical phylogenetic analysis resulted in the recognition of this assemblage as a paraphyletic gnathostome stem group[65–69]. Reconfiguration of the agnathan menagerie permitted reconstructions of evolutionary patterns in fin morphology and skeletal hard tissues, and identified the extinct jawless sister group of jawed vertebrates. Although many ostracoderm lineages have been considered contenders for this position, anatomical evidence overwhelmingly supports osteostracans. Like jawed vertebrates, but unlike other agnathans, osteostracans bear well-developed pectoral fins with associated girdles, a epicercal tail, and perichondral and cellular bone (Box 1).

The third and ongoing phase is the detailed scrutiny of the pioneering cladistic framework relating acanthodians and placoderms to modern jawed vertebrate lineages. Traction on this problem arose indirectly, beginning around the turn of the century with the development of expanded numerical phylogenetic analyses targeting relationships within osteichthyans[70–73] and chondrichthyans[74–76], but employing acanthodian and placoderm outgroups. These studies introduced the use of increasingly larger data sets, and provided the character information that would seed analyses targeting not individual lineages, but early jawed vertebrates as a whole. At the same time, a series of new fossil discoveries (outlined later) revealed unexpected anatomical combinations that raised serious questions about the coherence of acanthodians and placoderms. This set in motion a series of refined analyses of early jawed vertebrates bent on testing the supposed monophyly of these groups[42,49,58,77,78]. This final phase is a current debate and the setting for the following discussion.

New fossil discoveries and their importance

In this section, we highlight key finds since the 1980s that have challenged embedded perceptions and explain their importance in light of what is or was known about early jawed vertebrate evolution. Presented in approximate phylogenetic order, ascending from jawless members of the stem lineage, to placoderms, to members of the gnathostome crown, these discoveries provide a broad summary of the emerging picture of major evolutionary patterns in early gnathostomes. Detailed accounts of character transformation are provided elsewhere[20].

Shuyu and *Romundina* and their noses for success

The neurocranium, or braincase, is a primitively cartilaginous structure that houses the brain and paired sensory organs in vertebrates. When coated with a mineralized rind, structurally complex braincases can be

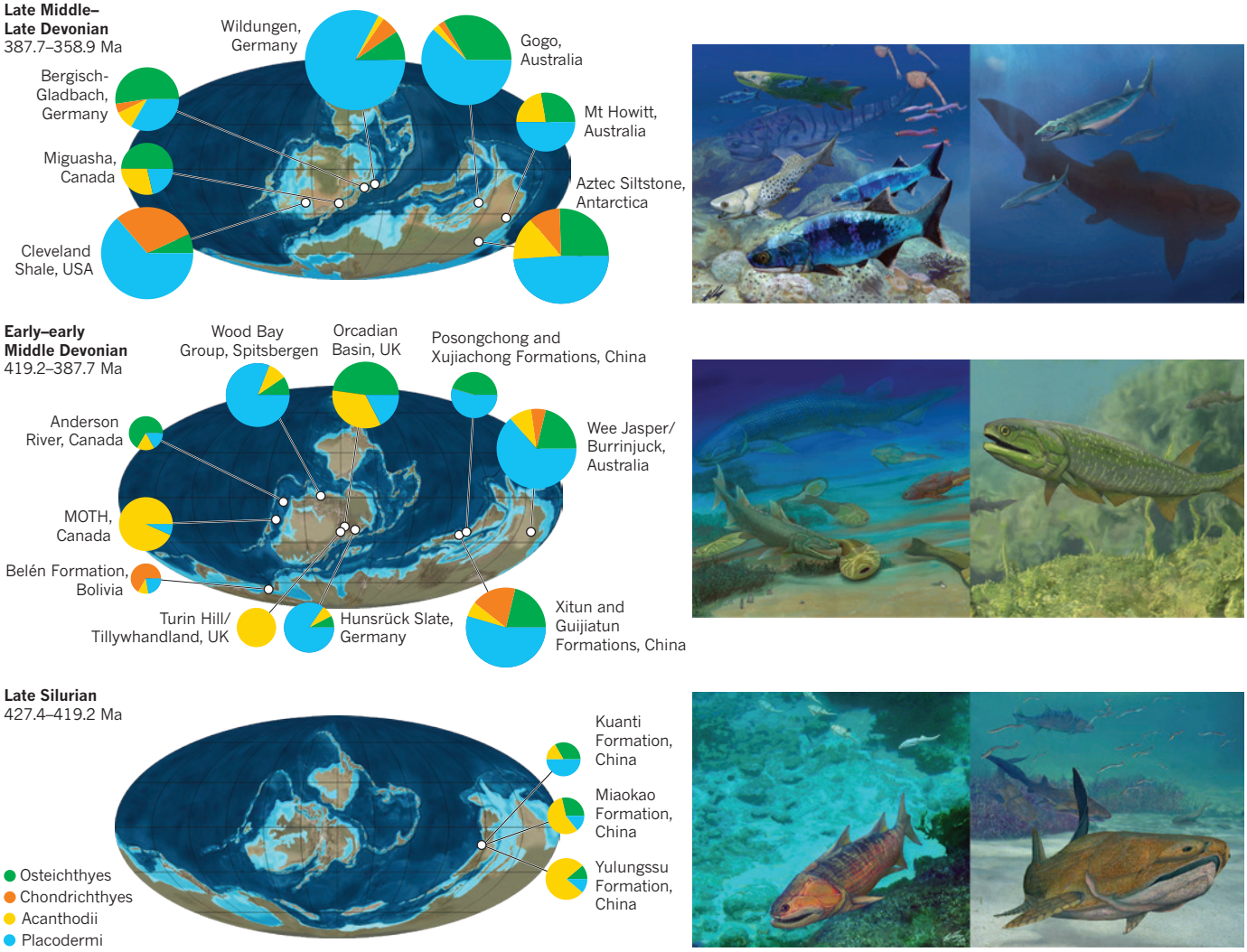


Figure 1 | Fossils relevant to early jawed-vertebrate evolution derive from major fossil sites in North America and Europe, and increasingly China and Australia. Palaeogeographic positions of localities bearing early jawed vertebrates and characterized by abundant fossils, high-fidelity preservation or both. Taxonomic breakdown of gnathostome diversity within sites is indicated by the associated pie charts and size-scaled to reported species richness. MOTH, Man on the Hill. The vignettes depict scenes based on key fossil sites: Gogo, Australia (left) and Cleveland Shale, USA (right) in the late Middle–Late Devonian; the Xitun Formation, China (left) and Orcadian Basin, UK (right) in the Early–early Middle Devonian; and the late Silurian Kuanti Formation, China (left and right). Illustrations by B. Choo, Flinders University. Palaeogeographic reconstructions by R. Blakey, Colorado Plateau Geosystems.

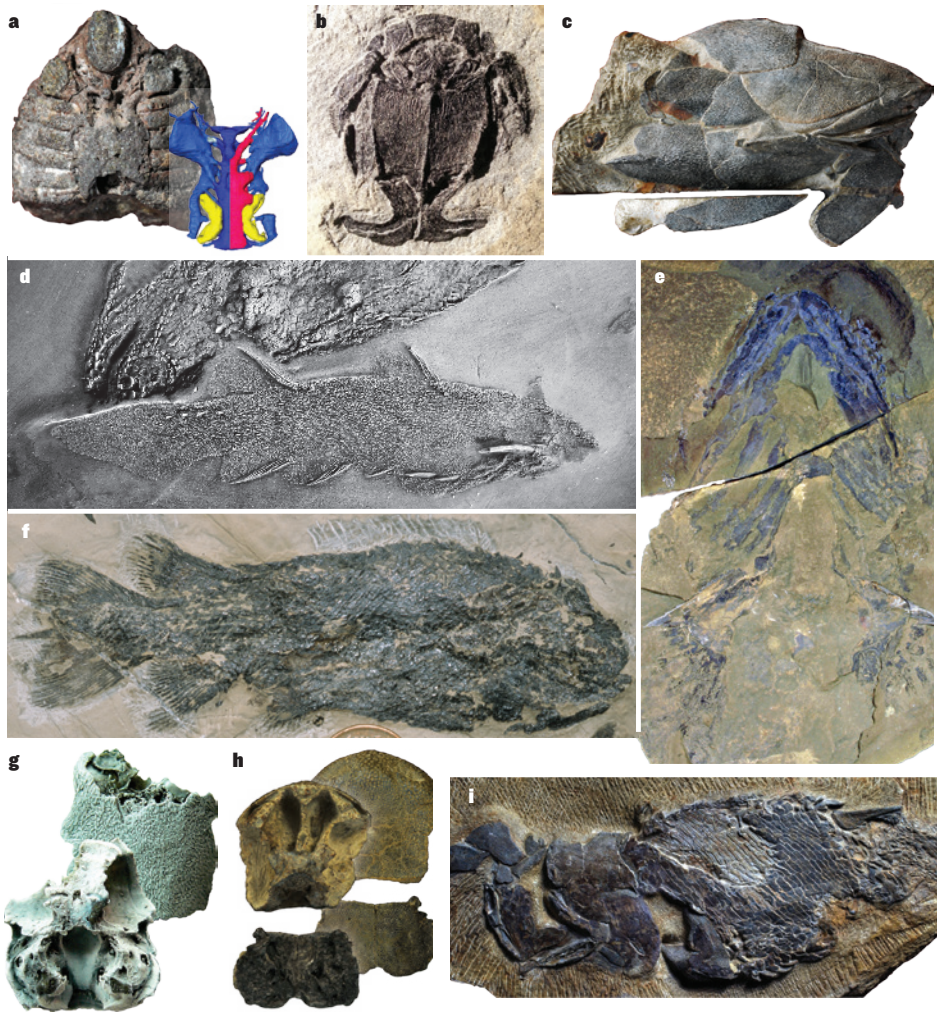


Figure 2 | Discoveries over the past two decades provide new clues about the evolution of early jawed vertebrates and their kin. **a**, High-fidelity virtual models of the Silurian galeaspid *Shuyu* reveal cranial architecture in jawless relatives of jawed vertebrates. **b**, Claspers in most placoderm groups, including antiarchs like *Microbrachius* shown here, raise questions about placoderm relationships and the evolution of vertebrate reproductive strategies. **c**, Osteichthyan-like pattern of bones in the Silurian placoderm *Entelognathus* suggest that the last common ancestor of all modern jawed vertebrates was clad in a bony-fish-like skeleton. **d**, Stunningly preserved fossils from the Early Devonian Man on the Hill (MOTH) locality of Canada challenges acanthodian monophyly, suggesting affinities with chondrichthyans. **e**, Pectoral-fin spines and tooth whorls with fused bases in the Early Devonian chondrichthyan *Doliodus* are features typically associated with acanthodians. **f**, The Early Devonian osteichthyan *Dialipina* shows a puzzling combination of traits despite being initially identified as a ray-finned fish based on isolated scales. **g**, An Early Devonian braincase attributed to the osteichthyan *Ligulalepis* shows features generally associated with placoderms and chondrichthyans. **h**, Braincase of *Psarolepis*, an Early Devonian lobe-finned osteichthyan from China represented by isolated bones, including spines of the kind associated with chondrichthyans, placoderms and acanthodians. **i**, The surprising reconstruction of *Psarolepis* was corroborated by the discovery of the more complete and even more ancient *Guiyu*, from the late Silurian of China. Images courtesy of **a**, Z. Gai; **b**, **g**, J. Long; **c**, **i**, M. Zhu; **e**, R. Miller; **f**, S. Cumbaa.

preserved as fossils and are a key source of phylogenetic information. Discriminating between specialized and primitive features in jawed vertebrates demands comparison with jawless fishes, but knowledge of the internal anatomy in ostracoderm lineages that lack endoskeletal mineralization is rudimentary[10,65]. By contrast, a thin coat of bone surrounds the cartilage forming the consolidated braincase and supports for the gills and pectoral fins of osteostracans. This permitted the first detailed reconstructions of osteostracan brains, cranial vessels and nerves nearly a century ago[50,51]. Galeaspids also bear a mineralized endoskeleton, but interpretations of their neurocranial structure have long been sketchy. High-resolution synchrotron scanning of the early galeaspid *Shuyu*[57] reinforced past identifications of widely separated, anterolaterally placed nasal capsules[68,79] that open medially into a central, dorsally directed duct that is also joined by the hypophysis (Fig. 2a). Thus, galeaspids show a tantalizing mosaic of cyclostome-like (nasal capsules located well behind the front of the head and opening into a common nasohypophyseal duct) and crown gnathostome-like (broad separation of nasal capsules) traits in the anterior region of the skull, and suggest that the cyclostome-like geometry of the better known osteostracans might be secondary. These features are more than just anatomical arcana — broad separation of nasal capsules is interpreted as a developmental necessity for the origin of jaws because the median nasohypophyseal placode of cyclostomes obstructs anterior growth of neural crest cells that contribute substantially to mandibles[24,57,80]. It seems that restructuring of the anterior portion of the head continued after the origin of jaws. Posteriorly placed, separate nasal capsules resembling those of galeaspids characterize the least crownward placoderms such as antiarchs, *Brindabellaspis* and *Romundina*, but these share with other jawed vertebrates a hypophysis that opens into the mouth, rather than a common nasohypophyseal duct as in agnathans[58]. By contrast, more crownward placoderms such as arthrodires, with their anteriorly placed nasal capsules, broadly resemble crown gnathostomes. These major architectural changes reflect a key piece of evidence for placoderm paraphyly[49,58,73,77,78], but ambiguities in the relationships among placoderms do not provide a consistent picture for the evolution of skull geometry in this crownward segment of the gnathostome stem.

Claspers and their evolutionary implications

The ptyctodontid placoderms have long been known to possess claspers[81], intromittent organs associated with the pelvic fins and evidence of internal fertilization. This trait factored in early cladistic investigations of placoderm intra- and interrelationships, tying placoderms to chondrichthyans[52] and fuelling arguments that ptyctodonts are the sister group of all other placoderms[10]. The discovery of arthrodire embryos within adult specimens prompted renewed investigation of this group in which long-overlooked evidence of claspers was finally discovered[82–84], followed by the realization that antiarchs also possessed these structures[85] (Fig. 2b). The palaeobiological and reproductive importance of claspers has been well considered[83,85], but their full phylogenetic importance is unresolved. Current phylogenetic consensus does not regard placoderm and chondrichthyan claspers to be homologous[20], but the homology of claspers within placoderms seems likely. Placoderm paraphyly demands the loss of internal fertilization before the origin of crown gnathostomes, signalling an unprecedented shift in reproductive biology within vertebrates[85]. Thus, we face two problematic alternatives: either internal fertilization was lost in a crownward segment of the gnathostome stem, defying observational data on the reproductive biology of living vertebrates[85], or placoderms with claspers form a clade, contradicting the apparent signal of other traits[58].

Entelognathus reframes ancestral conditions

The perceived 'primitiveness' of chondrichthyan anatomy entrenched in many general introductions to vertebrate biology has deep pre-Darwinian roots. Faced only with living species, this view seems reasonable enough: with their shagreen of tiny scales and cartilaginous internal skeletons, chondrichthyans seem to be tailor-made morphological intermediates between the naked hagfishes and lampreys on the one hand and the internally and externally bony osteichthyans on the other. The fossil record subverts this tidy picture by showing that both large dermal plates and a bony internal skeleton are innovations that arose long before the divergence of osteichthyans and chondrichthyans[22,66,67,69,86]. However, the condition of the skeleton in the last common ancestor of jawed vertebrates has remained controversial thanks to two mutually reinforcing phenomena: a reluctance to make explicit comparisons between the bony plates of osteichthyans and placoderms, and repeated interpretations of at least some acanthodians as early osteichthyan relatives[41,48,49,73,77]. Together these factors paint a picture of an ancestral crown gnathostome covered in a 'micromeric' outer skeleton of tiny scales, with a 'macromeric' skeleton composed of large plates reappearing in the osteichthyan lineage. This view was turned on its head by the discovery of the late Silurian *Entelognathus* in China[78] (about 423 Ma; Fig. 2c). Although *Entelognathus* broadly resembles a standard-issue placoderm, its cheek and upper and lower jaws are covered with bones that match the pattern seen in osteichthyans, rather than other placoderms. This remarkable correspondence suggests that there is evolutionary continuity between the large dermal plates of placoderms and those of bony fishes[42,58,78].

Man on the Hill brings acanthodians into the light

The Man on the Hill (MOTH) locality in the Northwest Territories of Canada is an Early Devonian (about 419 Ma) Konservat Lagerstätte yielding articulated early vertebrates. Originally discovered in the 1970s[64], new collections and advances in chemical preparation have since revealed exquisitely preserved fossils (Fig. 2d). Jawed vertebrates from MOTH are mostly acanthodians (Fig. 1), providing important anatomical detail on this enigmatic assemblage. Previously, the record of complete acanthodian fossils was mostly restricted to crudely prepared specimens from low-diversity, fluvial–lacustrine Early Devonian deposits of the United Kingdom[12]. By contrast, acid-prepared acanthodians from the species-rich marine MOTH locality reveal crisp anatomical details. In particular, a host of these species have umbellate and denticle-like scales such as those found in chondrichthyans[87–90]. Perhaps more importantly, the MOTH fauna include examples of acanthodian-like fishes covered in scales with growth patterns and structure previously known only from isolated fragments, but conventionally assigned to chondrichthyans[89]. This simultaneously suggests a position for acanthodians in the jawed vertebrate tree, while undermining confidence that they comprise a natural group.

The inside story on acanthodian morphology

Several early placoderms, osteichthyans and chondrichthyans yield detailed braincases[10,52,91], but acanthodian examples are rare. Subject to many re-interpretations over the past 100 years[12,48,49], the neurocranium of the Permian *Acanthodes* is central to debates on the evolutionary affinities of acanthodians. Various authors have been impressed by what they perceived as either particularly osteichthyan-like[41,48,77] or chondrichthyan-like[49,52] features of *Acanthodes*, triggering contrasting views on the placement of acanthodians as a whole. The Early Devonian (around 419 Ma) *Ptomacanthus* also preserves a braincase, although detail is obscure to the degree that this structure was initially ignored. Re-examination of *Ptomacanthus* revealed a neurocranium with a gross architecture that is more similar to that of placoderms or chondrichthyans than that of *Acanthodes* and osteichthyans, providing evidence in the first explicit argument for acanthodian paraphyly[77].

A sneak peek at early shark anatomy

With a sparse early record, interpretation of primitive chondrichthyan conditions drew heavily on body fossils from the latest Devonian[11] and even younger braincases[91], all of which are probably highly specialized. This changed with two stunning finds in the early 2000s. First was the discovery of more complete neurocrania of *Pucapampella* from the Early Devonian of Bolivia[76] and a similar South African form[92]. Previously named on the basis of an isolated neurocranial base, *Pucapampella* bears a chondrichthyan-specific hard tissue (prismatic calcified cartilage) in combination with a ventral fissure: a persistent division between two embryonic braincase components. Absent in ostracoderms, placoderms and other chondrichthyans, but present in *Acanthodes* and bony fishes, the ventral fissure was long considered key evidence for a close relationship between acanthodians and osteichthyans[48]. *Pucapampella* suggests that this trait is a general feature of crown-group gnathostomes. Subsequent discoveries provided additional anatomical details for *Pucapampella*, revealing peculiar teeth and jaws to accompany its unanticipated neurocranial architecture[34]. Hot on the heels of *Pucapampella* came the discovery of the oldest articulated chondrichthyan. *Doliodus*, from the Early Devonian of New Brunswick[93], was known for more than a century only by isolated teeth, and assigned to acanthodians. Recovery of an articulated head and forequarters revealed the signature chondrichthyan trait of prismatic calcified cartilage occurring in a fish with stubby spines along the leading edges of its pectoral fins (Fig. 2e), casting further doubt on acanthodian monophyly. Subsequent analysis of the braincase[59] and dentition[60,94] of *Doliodus* revealed primitive character states, such as fused tooth bases, not widely seen in crown chondrichthyans and certainly absent in modern sharks and rays, but common to acanthodians and early osteichthyans.

Rosetta stones for fragmentary bony fish remains

Fossil bony fishes have conventionally been deposited in one of the two living divisions: actinopterygians or sarcopterygians. This leaves the osteichthyan stem bereft of fossils that document the origin of this enormously successful clade. A series of isolated scales of late Silurian–Early Devonian age were loosely tethered to actinopterygians as their representatives[38,39], but the discovery of more complete material attributed to *Dialipina*[95] (Fig. 2f) and *Ligulalepis*[54,55] (Fig. 2g) raised questions about their actinopterygian affinities, and the importance of scale-based characters used to identify ray-finned fishes[41,73]. The braincase aligned with the scale-taxon *Ligulalepis* shows evidence of an eyestalk[54,55], a cartilaginous plinth that supports the eye in chondrichthyans and placoderms, but that is absent in modern osteichthyans. This might suggest *Ligulalepis* is a stem osteichthyan, but reports of eyestalks in early sarcopterygians[72] argue for parallel loss in the two bony fish divisions. Complete specimens of *Dialipina* are even more puzzling, marrying a tail geometry found only in lobe-finned fishes with a cheek comprising tiny bones that bear no clear resemblance to the large plates of other osteichthyans or even *Entelognathus*. *Ligulalepis* and *Dialipina* vacillate between Actinopterygii and the osteichthyan stem in many analyses[42,78], and solid placements are likely to be elusive until these taxa are more completely documented.

Psarolepis and *Guiyu* encapsulate the revolution

Perhaps more than any other discovery, *Psarolepis* represents the principal instigator of the current revolution in early jawed-vertebrate systematics. Recovered from late Silurian and earliest Devonian rocks of China, it is one of the earliest bony fishes (Fig. 1). First identified as a stem lungfish on the basis of jaw and braincase material[96], subsequent investigation of *Psarolepis* and the discovery of isolated cheek and shoulder bones highlighted more interesting affinities[70]. *Psarolepis* exhibits two hallmarks of the lobe-finned fishes: a braincase divided into front and hind units by an articulating joint and a pore-canal complex in its dermal bones (Fig. 2h). However, the cleaver-shaped cheek and maxilla (upper external jaw bone) bear an uncanny resemblance to those of early ray-finned fishes, suggestive of a shared primitive condition for bony fishes. More surprisingly, *Psarolepis* bristled with spines: the shoulder girdle bears a pronounced spine over the fin articulation area, reminiscent of acanthodians and some placoderms, whereas the dorsal fins were preceded by spines like those of chondrichthyans and acanthodians. *Psarolepis* is most reasonably interpreted as a

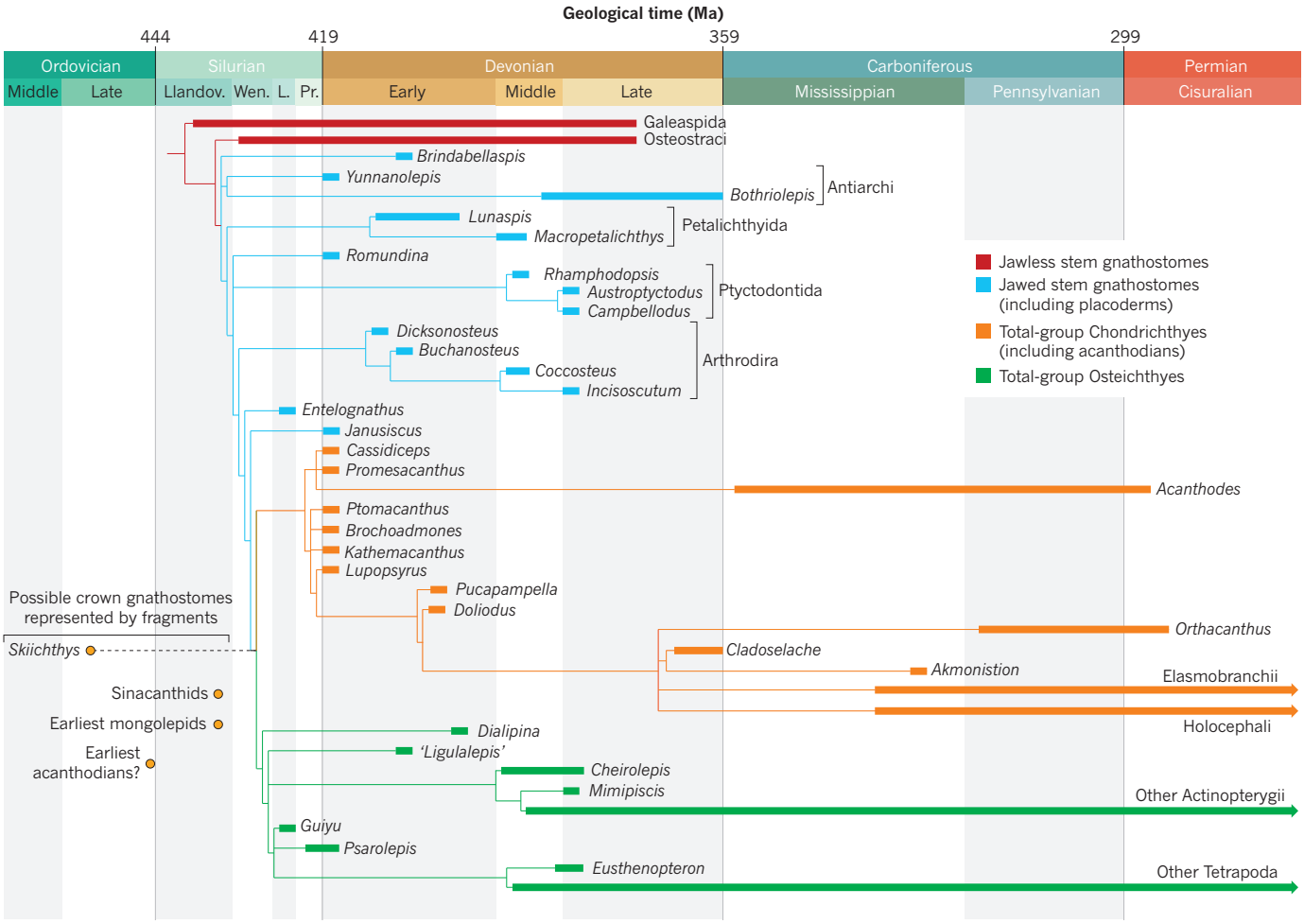


Figure 3 | Time-calibrated phylogeny of early jawed vertebrates and their immediate jawless relatives, showing minimum times of divergence based on fossil evidence. Topology based on ref. 42, with some taxa omitted for clarity and modifications showing presumed phylogenetic positions of key extant lineages. Also shown are key early jawed vertebrates or putative jawed vertebrates with uncertain affinities to the crown group. The minimum age of the gnathostome crown could be profoundly recalibrated if *Skiichthys*[28] is confirmed as a crown-group gnathostome. Dotted line indicates possible range extension for the gnathostome crown node. Llandov., Llandovery; Wen. Wenlock; L., Ludlow; Pr., Přídolí.

stem-group sarcopterygian[9,72,73], and thus an early example of the bony fish lineage that would give rise to tetrapods. However, it is held in this position by such a small number of traits, and retains so many plesiomorphies, that some analyses have recovered it as a stem-group osteichthyan[70,71]. This shook confidence in the seemingly stable, decades-old sets of attributes that characterize major early vertebrate groups[10]. However, the disarticulated nature of these fossils raised the troubling possibility that the combination of characters in *Psarolepis* was chimaeric: parts of different species misattributed to a single one. This concern was rejected, albeit indirectly, by the discovery of *Guiyu*[9] (Fig. 2j). Broadly similar to *Psarolepis,* but from even older Silurian rocks in China (about 423 Ma), *Guiyu* provides exceptional corroboration that traits such as a jointed braincase occurred in the same animal as pectoral- and dorsal-fin spines, and delivers further surprises, including the presence of placoderm-like external pelvic girdles[97]. Interpreted as an early sarcopterygian, *Guiyu* also shows that the last common ancestor of all modern osteichthyans arose no later than the Silurian, before the Devonian 'Age of Fishes'.

The re-shaping of early jawed vertebrate phylogeny

This panoply of new taxa and unexpected character distributions fuelled doubts about the status of classic early jawed vertebrate catagories[93,98], but early studies did not match these queries with cladistic tests. In the past five years, the field has witnessed a spate of numerical analyses giving rise to rapidly shifting perspectives on phylogenetic relationships[9,42,49,58,77,85]. However, some stable patterns are apparent and key areas of ongoing debate are now coming into focus.

The monophyly of fossil osteichthyans and chondrichthyans is universally supported. Placoderms are repeatedly recovered as stem-group gnathostomes and acanthodians are generally agreed to be members of the gnathostome crown, with some noteworthy exceptions[49]. Major differences with previous hypotheses stem from important shifts in approach, such as abandoning earlier assumptions of placoderm and acanthodian monophyly. In all cases so far, the monophyly of placoderms has been rejected and, in all but one[58], acanthodian monophyly has also been rejected.

In the earliest iterations, acanthodians were inferred to be massively paraphyletic, with some members associated with chondrichthyan, osteichthyan and gnathostome stem branches[49,58]. This configuration helped to explain the odd conjunction of osteichthyan, chondrichthyan and more primitive characters found in acanthodians. Furthermore, it implied an acanthodian-like appearance of the ancestral crown gnathostome: a small fusiform fish, covered in a denticle shagreen, a skull composed of mostly undifferentiated plates, with spines preceding the fins. The unfortunate complication of this hypothesis was that it implied non-homology of osteichthyan and placoderm armoured exoskeletons. Similarities between osteichthyan and placoderm skulls and shoulder girdles had not gone unnoticed[70,71,99], but were matched by dismissals citing 'fundamental differences' in construction[100]. The discovery of *Entelognathus* (already discussed) deals a blow to the latter perspective. Phylogenetic analysis accompanying the discovery[78] unsurprisingly led to a wholesale

shift of acanthodian-type taxa to the chondrichthyan total group. Every subsequent analysis has corroborated this outcome[42,58,85]. This key rearrangement eliminates the need to invoke convergence between placoderm and osteichthyan exoskeletons. By viewing the fragmented dermal skeletons of chondrichthyans and acanthodians as a derived condition, no special sister group relationship between osteichthyans and placoderms is implied, as had been assumed in the past[99].

Current analyses universally reject placoderm monophyly, with arthrodires (and similar forms such as *Entelognathus*) resolved closest to the gnathostome crown (Fig. 3). This arrangement suggests that resemblances between arthrodires and modern gnathostomes are homologous — a point reinforced by the arthrodire gestalt of *Entelognathus*. Likewise, it suggests that the similarities between the more flat-headed and presumably benthic placoderms, such as antiarchs and petalichthyids, and jawless outgroups reflect a shared primitive condition[10,20,77]. This has the convenient effect of stretching the placoderms into an array of jaw-bearing stem gnathostomes, although mandibles remain unknown in forms such as *Brindabellaspis* and petalichthyids.

The consistency of placoderm paraphyly across recent analyses[20,42,49,58,73,77,78,85] suggests that this is well supported. However, available solutions are not wholly independent, with each data set incrementally updated from a core original study[77]. Perhaps notably, the addition of taxa and characters has not increased support for the paraphyletic placoderm backbone. Instead, successive analyses have seen a winnowing of branch support for the deepest divergences among jaw-bearing stem gnathostomes, coupled with inconsistent arrangements of major placoderm lineages crownward of antiarchs and *Brindabellaspis*. This instability, combined with potential placoderm synapomorphies such as pelvic claspers[85] and a persistent fissure between the nasal capsules and the remainder of the braincase[20], indicate that the 'placoderm problem' is far from resolved. A satisfactory resolution of the relationships of placoderms will have profound consequences for our understanding of the origin of modern jawed vertebrates.

Future directions

Early jawed vertebrate phylogenetics is in a state of infancy, but rapid progress is being made. Present discourse on early jawed vertebrate phylogenetics is marked by a growth of healthy debate and a relative lack of the kind of dogmatism that held back the field for nearly half a century. The question of the origin of the jaws themselves remains open. So far, the problem has been debated in terms of highly idealized archetypal scenarios, such as the transformation of gill arches into jaws[1]. From both palaeontological and neontological perspectives, this scenario has proved deficient[6,10,80]. Little direct evidence of the visceral skeleton of fossil jawless fishes is known; even the proximate outgroups of the jawed vertebrates — osteostracans and galeaspids — are presumed to have been jawless, but remains of the oral skeleton remain absent. What is known of the oral regions of osteostracans and galeaspids suggests that they possessed mouths that were specialized relative to the branchial arches, a condition consistent with modern jawless fishes[10]. Placoderm paraphyly raises some hope that relevant data could be sourced from this assemblage (for example, *Brindabellaspis* or petalichthyids). The discovery of additional fossils will hopefully help to fill these gaps, but they will not be sufficient by themselves. Rigorous phylogenetic analysis must accompany these new finds to avoid simply shoehorning fossils into appealing narratives[12]. ■

Received 6 November 2014; accepted 9 February 2015.

1. Gegenbaur, C., Bell, F. J. & Lankester, E. R. *Elements of Comparative Anatomy* (Macmillan and Co., 1878).
2. Balfour, F. M. On the development of the skeleton of the paired fins of Elasmobranchii, considered in relation to its bearings on the nature of the limbs of the Vertebrata. *Proc. Zool. Soc. Lond.* **49,** 656–670 (1881).
3. de Beer, G. *The Development of the Vertebrate Skull* (Oxford Univ. Press, 1937).
4. Reif, W.-E. Evolution of dermal skeleton and dentition in vertebrates. *Evol. Biol.* **15,** 287–368 (1982).
5. Shubin, N. H. Origin of evolutionary novelty: examples from limbs. *J. Morphol.* **252,** 15–28 (2002).
6. Kuratani, S. Evolution of the vertebrate jaw: comparative embryology and molecular developmental biology reveal the factors behind evolutionary novelty. *J. Anat.* **205,** 335–347 (2004).
7. Shigetani, Y., Sugahara, F. & Kuratani, S. A new evolutionary scenario for the vertebrate jaw. *Bioessays* **27,** 331–338 (2005).
8. Wagner, G. P. & Lynch, V. J. Evolutionary novelties. *Curr. Biol.* **20,** R48–R52 (2010).
9. Zhu, M. *et al*. The oldest articulated osteichthyan reveals mosaic gnathostome characters. *Nature* **458,** 469–474 (2009).
10. Janvier, P. *Early Vertebrates* (Clarendon, 1996).
This masterful summary provides a window on the 'state of the art' immediately preceding the major changes to our understanding of relationships among early gnathostomes that took place over the past two decades, and is still an indispensible and accessible resource.
11. Dean, B. Contributions to the morphology of *Cladoselache* (*Cladodus*). *J. Morphol.* **9,** 87–114 (1894).
12. Watson, D. M. S. The acanthodian fishes. *Philos. Trans. R. Soc. Lond.* **228,** 49–146 (1937).
13. Zangerl, R. & Williams, M. E. New evidence on the nature of the jaw suspension in Palaeozoic anacanthous sharks. *Palaeontology* **18,** 333–341 (1975).
14. Gregory, W. K. Further observations on the pectoral girdle and fin of *Sauripterus taylori* Hall, a crossopterygian fish from the Upper Devonian of Pennsylvania, with special reference to the origin of the pentadactylate extremities of Tetrapoda. *Proc. Am. Phil. Soc.* **75,** 673–690 (1935).
15. Miles, R. S. A reinterpretation of the visceral skeleton of *Acanthodes*. *Nature* **204,** 457–459 (1964).
16. Davis, M. C., Shubin, N. & Daeschler, E. B. A new specimen of *Sauripterus taylori* (Sarcopterygii, Osteichthyes) from the Famennian Catskill Formation of North America. *J. Vertebr. Paleontol.* **24,** 26–40 (2004).
17. Kemp, T. S. *The Origin and Evolution of Mammals* (Oxford Univ. Press, 2005).
18. Makovicky, P. J. & Zanno, L. E. in *Living Dinosaurs: The Evolutionary History of Modern Birds* (eds Dyke, G. & Kaiser, G.) 9–29 (Wiley, 2011).
19. Clack, J. A. *Gaining Ground* (Indiana Univ. Press, 2012).
20. Brazeau, M. D. & Friedman, M. The characters of Palaeozoic jawed vertebrates. *Zool. J. Linn. Soc.* **170,** 779–821 (2014).
21. Chen, M., Zou, M., Yang, L. & He, S. Basal jawed vertebrate phylogenomics using transcriptomic data from Solexa sequencing. *PLoS ONE* **7,** e36256 (2012).
22. Donoghue, P. C., Forey, P. L. & Aldridge, R. J. Conodont affinity and chordate phylogeny. *Biol. Rev. Camb. Philos. Soc.* **75,** 191–251 (2000).
23. Ota, K. G., Fujimoto, S., Oisi, Y. & Kuratani, S. Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. *Nature Commun.* **2,** 373 (2011).
24. Oisi, Y., Ota, K. G., Kuraku, S., Fujimoto, S. & Kuratani, S. Craniofacial development of hagfishes and the evolution of vertebrates. *Nature* **493,** 175–180 (2013).
25. Ota, K. G., Kuraku, S. & Kuratani, S. Hagfish embryology with reference to the evolution of the neural crest. *Nature* **446,** 672–675 (2007).
26. Heimberg, A. M., Cowper-Sal-lari, R., Semon, M., Donoghue, P. C. & Peterson, K. J. microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. *Proc. Natl Acad. Sci. USA* **107,** 19379–19383 (2010).
27. Karatajute-Talimaa, V. & Predtechenskyj, N. The distribution of the vertebrates in the Late Ordovician and Early Silurian palaeobasins of the Siberian Platform. *Bull. Mus. Natl Hist. Nat.* **4,** 39–55 (1995).
28. Smith, M. M. & Sansom, I. J. Exoskeletal micro-remains of an Ordovician fish from the Harding Sandstone of Colorado. *Palaeontology* **40,** 645–658 (1997).
29. Sansom, I. J., Davies, N. S., Coates, M. I., Nicoll, R. S. & Ritchie, A. Chondrichthyan-like scales from the Middle Ordovician of Australia. *Palaeontology* **55,** 243–247 (2012).
30. Zhao, W.-J. & Zhu, M. Siluro-Devonian vertebrate biostratigraphy and biogeography of China. *Palaeoworld* **19,** 4–26 (2010).
31. Anderson, P. S., Friedman, M., Brazeau, M. D. & Rayfield, E. J. Initial radiation of jaws demonstrated stability despite faunal and environmental change. *Nature* **476,** 206–209 (2011).
32. Karatajute-Talimaa, V. N., Novtistkaya, L. I., Rozman, K. S. & Sodov, J. *Mongolepis*, a new genus of Elasmobranchii from the Lower Silurian of Mongolia. *Paleontologicheskii zhurnal* **1,** 76–86 (1990).
33. Sansom, I. J., Wang, N.-Z. & Smith, M. The histology and affinities of sinacanthid fishes: primitive gnathostomes from the Silurian of China. *Zool. J. Linn. Soc.* **144,** 379–386 (2005).
34. Janvier, P. & Maisey, J. G. in *Morphology, Phylogeny and Paleobiogeography of Fossil Fishes* (eds Elliott, D. K., Maisey, J. G., Yu, X. & Miao, D.) 431–459 (Dr Freidrich Pfeil, 2010).
35. Panchen, A. L. & Smithson, T. R. Character diagnosis, fossils and the origin of tetrapods. *Biol. Rev. Camb. Philos. Soc.* **62,** 341–436 (1987).
36. Ahlberg, P. E. & Johanson, Z. Osteolepiforms and the ancestry of tetrapods. *Nature* **395,** 792–794 (1998).
37. Lukševičs, E., Lebedev, O. A. & Zakharenko, G. V. Palaeozoogeographical connections of the Devonian vertebrate communities of the Baltica Province. Part I. Eifelian-Givetian. *Palaeoworld* **19,** 94–107 (2010).
38. Schultze, H.-P. Palaeoniscoidea-Schuppen aus dem Unterdevon Australiens und Kanadas und aus dem Mitteldevon Spitzbergens [in German]. *British Mus. Nat. Hist. Geol.* **16,** 343–376 (1968).
39. Gross, W. Fragliche Actinopterygier-Schuppen aus dem Silur Gotlands [in German]. *Lethaia* **1,** 184–218 (1968).
40. Botella, H., Blom, H., Dorka, M., Ahlberg, P. E. & Janvier, P. Jaws and teeth of the earliest bony fishes. *Nature* **448,** 583–586 (2007).
41. Friedman, M. & Brazeau, M. D. A reappraisal of the origin and basal radiation of the Osteichthyes. *J. Vertebr. Paleontol.* **30,** 36–56 (2010).

42. Giles, S., Friedman, M. & Brazeau, M. D. Osteichthyan-like cranial conditions in an Early Devonian stem gnathostome. *Nature* http://dx.doi.org/10.1038/nature14065 (2015).
43. Märss, T., Turner, S. & Karatajute-Talimaa, V. in *Handbook of Paleoichthyology* Vol. 1B (ed. Schultze, H.-P.) (Dr Friedrich Pfeil, 2007).
44. Zhu, M. & Gai, Z.-K. Phylogenetic relationships of galeaspids (Agnatha). *Vertebr. PalAsiat.* **44,** 1–27 (2006).
45. Sansom, R. S. Endemicity and palaeobiogeography of the Osteostraci and Galeaspida: a test of scenarios of gnathostome evolution. *Palaeontology* **52,** 1257–1273 (2009).
46. Sansom, R. S. Phylogeny, classification and character polarity of the Osteostraci (Vertebrata). *J. Syst. Paleontol.* **7,** 95–115 (2009).
47. Young, G. C. Placoderms (armoured fish): dominant vertebrates of the Devonian period. *Annu. Rev. Earth Planet. Sci.* **38,** 523–550 (2010).
48. Miles, R. S. in *Interrelationships of Fishes* (eds Greenwood, P. H., Miles, R. S. & Patterson, C.) 63–103 (Academic, 1973).
This first-generation application of cladistic methodology to early jawed vertebrates placed the 'spiny sharks' as early relatives of bony fishes, a perspective that profoundly influenced perceptions of the ancestral crown gnathostome for more than 40 years.
49. Davis, S. P., Finarelli, J. A. & Coates, M. I. *Acanthodes* and shark-like conditions in the last common ancestor of modern gnathostomes. *Nature* **486,** 247–250 (2012).
50. Stensiö, E. A. The Devonian and Downtonian vertebrates of Spitsbergen. Part 1. Family Cephalaspidae. *Skr. Svalbard Ishav.* **12,** 1–391 (1927).
51. Stensiö, E. A. *The Cephalaspids of Great Britain* (British Museum (Natural History), 1932).
52. Jarvik, E. *Basic Structure and Evolution of Vertebrates* (Academic, 1980).
53. White, E. I. The larger arthrodiran fishes from the area of the Burrinjuck Dam, N.S.W. *Tran. Zoo. Soc. Lond.* **34,** 149–262 (1978).
54. Basden, A. M. & Young, G. C. A primitive actinopterygian neurocranium from the Early Devonian of Southeastern Australia. *J. Vertebr. Paleontol.* **21,** 754–766 (2001).
55. Basden, A. M., Young, G. C., Coates, M. I. & Richtie, A. The most primitive osteichthyan braincase? *Nature* **403,** 185–188 (2000).
56. Young, G. C. A new Early Devonian placoderm from New South Wales, Australia, with a discussion of placoderm phylogeny. *Palaeontogr. A* **167,** 10–76 (1980).
57. Gai, Z., Donoghue, P. C., Zhu, M., Janvier, P. & Stampanoni, M. Fossil jawless fish from China foreshadows early jawed vertebrate anatomy. *Nature* **476,** 324–327 (2011).
58. Dupret, V., Sanchez, S., Goujet, D., Tafforeau, P. & Ahlberg, P. E. A primitive placoderm sheds light on the origin of the jawed vertebrate face. *Nature* **507,** 500–503 (2014).
59. Maisey, J. G., Miller, R. & Turner, S. The braincase of the chondrichthyan *Doliodus* from the Lower Devonian Campbellton Formation of New Brunswick, Canada. *Acta Zool.* **90** (Suppl. 1), 109–122 (2009).
60. Maisey, J. G., Turner, S., Naylor, G. J. & Miller, R. F. Dental patterning in the earliest sharks: implications for tooth evolution. *J. Morphol.* **275,** 586–596 (2014).
61. Schaeffer, B. in *Problèmes Actuels de Paléontologie: Evolution des Vertébrés* Vol. 218 [in French] (ed. Lehman, J.-P.) 101–109 (Colloques internationaux du Centre national de la Recheche scientifique, 1975).
62. Long, J. A. & Trinajstic, K. The Late Devonian Gogo Formation Lägerstatte of Western Australia: exceptional early vertebrate preservation and diversity. *Annu. Rev. Earth Planet. Sci.* **38,** 255–279 (2010).
63. Zhu, M. Catalogue of Devonian vertebrates in China, with notes on bio-events. *Cour. Forsch. Inst. Senckenberg* **223,** 379–390 (2000).
64. Bernacsek, G. M. & Dineley, D. L. New acanthodians from the Delorme Formation (Lower Devonian) of N.W.T. Canada. *Palaeontogr. A* **159,** 1–25 (1977).
65. Janvier, P. & Blieck, A. New data on the internal anatomy of the Heterostraci (Agnatha), with general remarks on the phylogeny of the Craniota. *Zool. Scr.* **8,** 287–296 (1979).
66. Janvier, P. The phylogeny of Craniata, with particular reference to the significance of fossil 'agnathans'. *J. Vertebr. Paleontol.* **1,** 121–159 (1981).
This article established osteostracans and galeaspids as successive outgroups to, and thus important comparative models for, jawed vertebrates, an arrangement that has survived intact for more than three decades.
67. Forey, P. L. Yet more reflections on agnathan-gnathostome relationships. *J. Vertebr. Paleontol.* **4,** 330–343 (1984).
68. Wang, N.-Z. in *Early Vertebrates and Related Problems of Evolutionary Biology* (eds Chang, M.-M., Lui, Y.-H. & Zhang, G.-R.) (Science, 1991).
69. Forey, P. L. & Janvier, P. Agnathans and the origin of jawed vertebrates. *Nature* **361,** 129–134 (1993).
70. Zhu, M., Yu, X. & Janvier, P. A primitive fossil fish sheds light on the origin of bony fishes. *Nature* **397,** 607–610 (1999).
The bizarre combination of traits for *Psarolepis* reported in this article highlighted weaknesses in existing phylogenies of early jawed vertebrates, and triggered a resurgence in systematic studies.
71. Zhu, M. & Schultze, H.-P. in *Major Events in Early Vertebrate Evolution* (ed. Ahlberg, P. E.) 81–84 (Taylor & Francis, 2001).
72. Zhu, M., Yu, X. & Ahlberg, P. E. A primitive sarcopterygian fish with an eyestalk. *Nature* **410,** 81–84 (2001).
73. Friedman, M. *Styloichthys* as the oldest coelacanth: implications for early osteichthyan interrelationships. *J. Syst. Palaeontology* **5,** 289–343 (2007).
74. Coates, M. I. & Sequiera, S. E. K. A new stethacanthid chondrichthyan from the Lower Carboniferous of Bearsden, Scotland. *J. Vertebr. Paleontol.* **21,** 438–459 (2001).
75. Coates, M. I. & Sequiera, S. E. K. in *Major Events in Early Vertebrate Evolution* (ed. Ahlberg, P. E.) 241–262 (Taylor & Francis, 2001).
76. Maisey, J. G. in *Major Events in Early Vertebrate Evolution* (ed. Ahlberg, P. E.) 263–288 (Taylor & Francis, 2001).
77. Brazeau, M. D. The braincase and jaws of a Devonian 'acanthodian' and modern gnathostome origins. *Nature* **457,** 305–308 (2009).
This study was the first to rigorously test — and, in doing so, to reject — placoderm and acanthodian monophyly, and provides the empirical core for most subsequent phylogenetic investigations of early gnathostomes.
78. Zhu, M. *et al*. A Silurian placoderm with osteichthyan-like marginal jaw bones. *Nature* **502,** 188–193 (2013).
Of the many remarkable early gnathostome fossils to emerge from China, few have shifted the evolutionary paradigm as much as *Entelognathus*, a placoderm-like creature with jaw bones resembling those of bony fishes.
79. Halstead, L. B. Internal anatomy of the polybranchiaspids (Agnatha, Galeaspida). *Nature* **282,** 833–836 (1979).
80. Kuratani, S. Evolution of the vertebrate jaw from developmental perspectives. *Evol. Dev.* **14,** 76–92 (2012).
81. Miles, R. S. Observations on the ptyctodont fish, *Rhamphodopsis* Watson. *Zool. J. Linn. Soc.* **47,** 99–120 (1967).
82. Ahlberg, P., Trinajstic, K., Johanson, Z. & Long, J. Pelvic claspers confirm chondrichthyan-like internal fertilization in arthrodires. *Nature* **460,** 888–889 (2009).
This direct evidence of claspers in arthrodires renewed the palaeobiological importance of placoderms regarding internal fertilization, but potentially weakens the case for their paraphyly.
83. Trinajstic, K., Boisvert, C., Long, J., Maksimenko, A. & Johanson, Z. Pelvic and reproductive structures in placoderms (stem gnathostomes). *Biol. Rev. Camb. Philos. Soc.* http://dx.doi.org/10.1111/brv.12118 (2014).
84. Long, J. A., Trinajstic, K. & Johanson, Z. Devonian arthrodire embryos and the origin of internal fertilization in vertebrates. *Nature* **457,** 1124–1127 (2009).
85. Long, J. A. *et al*. Copulation in antiarch placoderms and the origin of gnathostome internal fertilization. *Nature* **517,** 196–199 (2015).
86. Janvier, P. The relationships of the Osteostraci and Galeaspida. *J. Vertebr. Paleontol.* **4,** 344–358 (1984).
87. Hanke, G. F. & Wilson, M. V. H. in *Recent Advances in the Origin and Early Radiation of Vertebrates* (eds Arratia, G., Wilson, M. V. H. & Cloutier, R.) 189–216 (Dr Friedrich Pfeil, 2004).
88. Hanke, G. F. & Wilson, M. V. H. in *Morphology, Phylogeny and Paleobiogeography of Fossil Fishes* (eds Elliott, D. K., Maisey, J. G., Yu, X. & Miao, D.) 149–182 (Dr Friedrich Pfeil, 2010).
89. Hanke, G. F., Wilson, M. V. H. & Saurette, F. Partial articulated specimen of the Early Devonian putative chondrichthyan *Polymerolepis whitei* Karatajūtė-Talimaa, 1968, with an anal fin spine. *Geodiversitas* **35,** 529–543 (2013).
90. Hanke, G. F. & Wilson, M. V. H. Anatomy of the Early Devonian acanthodian *Brochoadmones milesi* based on nearly complete body fossils, with comments on the evolution and development of paired fins. *J. Vertebr. Paleontol.* **26,** 526–537 (2006).
91. Schaeffer, B. The xenacanth shark neurocranium, with comments on elasmobranch monophyly. *Bull. Am. Mus. Nat. Hist.* **169,** 1–66 (1981).
92. Maisey, J. G. & Anderson, M. E. A primitive chondrichthyan braincase from the Early Devonian of South Africa. *J. Vertebr. Paleontol.* **21,** 702–713 (2001).
93. Miller, R. F., Cloutier, R. & Turner, S. The oldest articulated chondrichthyan from the Early Devonian period. *Nature* **425,** 501–504 (2003).
This reports the oldest record of an articulated chondrichthyan and the first example with paired fin spines, initiating the dissolution of support for acanthodian monophyly.
94. Turner, S. in *Recent Advances in the Origin and Early Radiation of Vertebrates* (eds Arratia, G., Wilson, M. V. H. & Cloutier, R.) 67–94 (Dr Friedrich Pfeil, 2004).
95. Schultze, H.-P. & Cumbaa, S. L. in *Major Events in Early Vertebrate Evolution* (ed. Ahlberg, P. E.) 315–332 (Taylor & Francis, 2001).
96. Yu, X. A new porolepiform-like fish, *Psarolepis romeri*, gen. et sp. nov. (Sarcopterygii, Osteichthyes) from the Lower Devonian of Yunnan, China. *J. Vertebr. Paleontol.* **18,** 261–274 (1998).
97. Zhu, M. *et al*. Fossil fishes from China provide first evidence of dermal pelvic girdles in osteichthyans. *PLoS ONE* **7,** e35103 (2012).
98. Coates, M. I. The evolution of paired fins. *Theory Biosci.* **122,** 266–287 (2003).
99. Gardiner, B. G. The relationships of placoderms. *J. Vertebr. Paleontol.* **4,** 375–395 (1984).
100. Young, G. C. The relationships of the placoderm fishes. *Zool. J. Linn. Soc.* **88,** 1–57 (1986).
This article provided an explicit argument for the status of placoderms as stem gnathostomes that has not been seriously challenged in the following three decades.

Acknowledgements This work was supported by the Philip Leverhulme Prize and John Fell Fund, both to M.F., and the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement number 311092 to M.D.B. Both authors contributed equally to this Review.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this paper at go.nature.com/snrujd. Correspondence should be addressed to M.B (m.brazeau@imperial.ac.uk) or M.F. (mattf@earth.ox.ac.uk).