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EARLY VERTEBRATE EVOLUTION

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Abstract: Debate over the origin and evolution of vertebrates has occupied biologists and palaeontologists alike for centuries. This debate has been refined by molecular phylogenetics, which has resolved the place of vertebrates among their invertebrate chordate relatives, and that of chordates among their deuterostome relatives. The origin of vertebrates is characterized by wide-ranging genomic, embryologic and phenotypic evolutionary change. Analyses based on living lineages suggest dramatic shifts in the tempo of evolutionary change at the origin of vertebrates and gnathostomes, coincident with whole-genome duplication events. However, the enriched perspective provided by the fossil record demonstrates that these apparent bursts of anatomical evolution and taxic richness are an artefact of the extinction of phylogenetic intermediates whose fossil remains evidence the gradual assembly of crown gnathostome characters in particular. A more refined understanding of the timing, tempo and mode of early vertebrate evolution rests with: (1) better genome assemblies for living cyclostomes; (2) a better understanding

of the anatomical characteristics of key fossil groups, especially the anaspids, thelodonts, galeaspids and pituriaspids; (3) tests of the monophyly of traditional groups; and (4) the application of divergence time methods that integrate not just molecular data from living species, but also morphological data and extinct species. The resulting framework will provide for rigorous tests of rates of character evolution and diversification, and of hypotheses of long-term trends in ecological evolution that themselves suffer for lack of quantitative functional tests. The fossil record has been silent on the nature of the transition from jawless vertebrates to the jawed vertebrates that have dominated communities since the middle Palaeozoic. Elucidation of this most formative of episodes likely rests with the overhaul of early vertebrate systematics that we propose, but perhaps more fundamentally with fossil grades that await discovery.

Key words: deuterostome, chordate, vertebrate, gnathostome, evolution, fossil record.

THE origin of vertebrates is widely appreciated as one of the most formative events in evolutionary history. The gulf is so large between those blessed with a backbone and their spineless relatives which have not, that the invertebrate–vertebrate divide is one of the most fundamental in biology, discriminating teaching, research, journals and conferences. To some great extent, this may be perceived as entirely arbitrary and a consequence of undue anthropocentrism. And yet, the origin of vertebrates is associated with a fundamental embryological revolution and the doubling or quadrupling of the genome. Without doubt, these profound changes helped vertebrates get ahead and, what is more, to get a head.

A PERSPECTIVE ON EARLY VERTEBRATE EVOLUTION BASED ON THE LIVING

To obtain an understanding of vertebrate origins, it is necessary to establish first their phylogenetic context

(Fig. 1). Their closest living relatives are the tunicates (Fig. 2A–B), together with which they comprise the clade Olfactores, to which the cephalochordates (Fig. 2D) are a sister lineage (Delsuc *et al.* 2006). All three comprise the chordate phylum which, together with the echinoderms and hemichordates (Ambulacraria), and perhaps the acoelomorph flatworms and *Xenoturbella*, comprise the deuterostomes (Philippe *et al.* 2011). Vertebrates inherit bilateral symmetry, a centralized nervous system and a perforated pharynx from the deuterostome crown ancestor, segmented musculature and a notochord from the chordate crown ancestor, and the rudiments of cranial sense organs and a fourth germ layer (neural crest) from the crown ancestor of Olfactores. These are major innovations, but they are merely the chassis onto which is bolted, in evolution and development, the fundamental novelties that distinguish vertebrates.

Vertebrate novelties can be discriminated as those features that vertebrates share, that are absent from their nearest living invertebrate relatives, the tunicates and the cephalochordates. The three principal lineages of

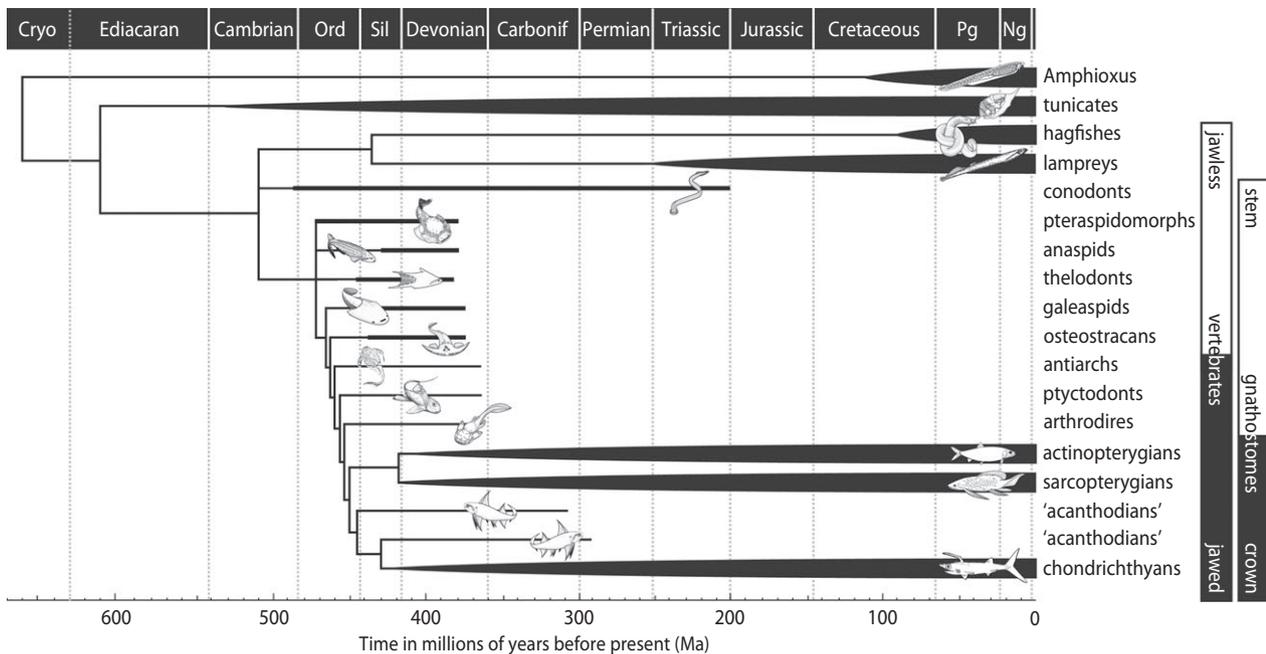


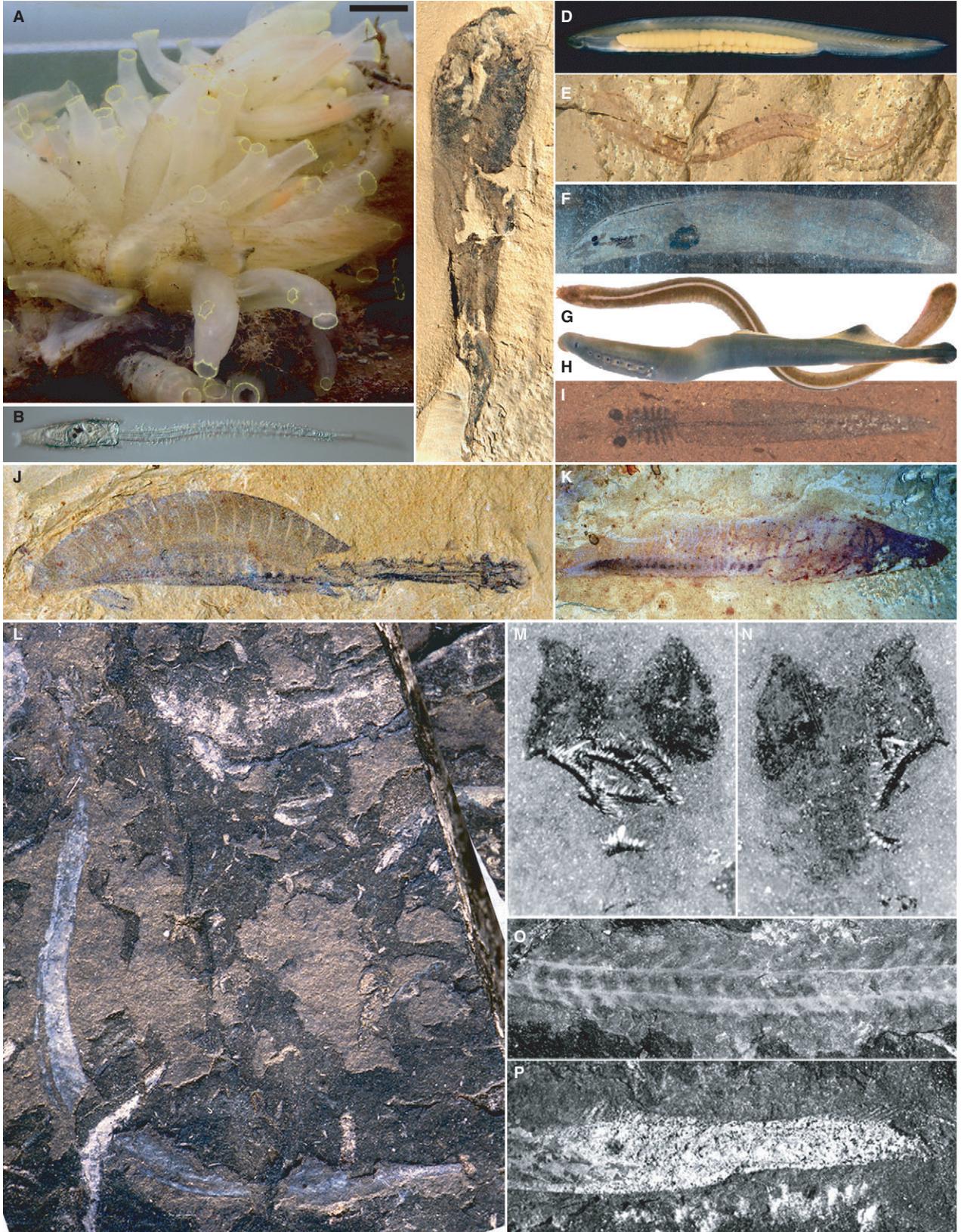
FIG. 1. Phylogeny of living chordates and a representative selection of extinct vertebrates plotted against geological time. Relationships are based upon Donoghue *et al.* (2000), Heimberg *et al.* (2010), Sansom *et al.* (2010) and Zhu *et al.* (2013). The spindles (dark grey) reflect the crown group divergence of the respective lineages: amphioxus (Nohara *et al.* 2004); tunicates (Swalla and Smith 2008); hagfishes and lampreys (Kuraku and Kuratani 2006); chondrichthyans (Inoue *et al.* 2010); and actinopterygians (Inoue *et al.* 2003). Node dates, where applicable, correspond with divergence estimates from Erwin *et al.* (2011). Geological timescale based on Gradstein *et al.* (2012). *Abbreviations:* Cryo, Cryogenian; Ord, Ordovician; Sil, Silurian; Carbonif, Carboniferous; Pg, Palaeogene; Ng, Neogene.

vertebrates are the jawless hagfishes and lampreys (Fig. 2G–H), and the jawed gnathostomes. There has been a long-standing debate over the relationships of these three lineages, which impacts on this exercise, viz. whether the lampreys are more closely related to hagfishes (cyclostome monophyly) or gnathostomes (cyclostome paraphyly). This debate appears to have lurched in favour of cyclostome monophyly (Fig. 1), revealing the ancestral crown vertebrate to have been more complex than either

hagfishes or lampreys, both of which lineages have lost vertebrate characters (Heimberg *et al.* 2010).

Thus, vertebrate novelties include paired optic, otic and nasal organs, a sensory line system, multifarious neurons and glia, a differentiated brain, braincase, a sympathetic nervous system, diverse pigment cells, cartilage, connective tissue, an adaptive immune system, a post anal tail, fin rays, arcualia, and gills, among very many other characters. The majority of these features are not unrelated,

FIG. 2. Soft-bodied invertebrate chordates and vertebrates. A–B, the tunicate *Ciona intestinalis* adult (A) and larva (B). C, *Shankouclava shankouense* (SK01001), a putative tunicate from the Cambrian Chengjiang Lagerstätten of Kunming, Yunnan Province, South China. D, *Branchiostoma lanceolata* adult male. E, *Cathaymyrus diadexus* (NWU 95-1405), a putative cephalochordate from the Cambrian Chengjiang Lagerstätten of Kunming, Yunnan Province, South China. F, *Myxinikela siroka* (NEIU MCP 126), a putative hagfish from the Pennsylvanian (Carboniferous) Mazon Creek Lagerstätten of Illinois, USA. G, The inshore hagfish *Eptatretus burgeri* in dorsal aspect. H, the Arctic lamprey *Lethenteron camtschaticum* in lateral aspect. I, *Mayomyzon pieckoensis* (FMNH PF 8167), a lamprey from the Pennsylvanian (Carboniferous) Mazon Creek Lagerstätten of Illinois, USA. J, *Haikouella lanceolata* (EC00213a), a putative chordate that has been assigned to multifarious deuterostome (and nondeuterostome) clades, from the Cambrian Chengjiang Lagerstätten of Kunming, Yunnan Province, South China. K, *Haikouichthys ercaicunensis* (YKLP (RCCBYU)-00195), a total-group vertebrate from the Cambrian Chengjiang Lagerstätten of Kunming, Yunnan Province, South China. L–P, IGSE 13821 *Clydagnathus winsorensis*, a conodont (total-group vertebrate) from the Mississippian (early Carboniferous) of Granton, Scotland; M–N, part and counterpart of the head showing paired structures interpreted as eyes, as well as the characteristic phosphatic elements comprising the feeding apparatus; O, detail of trunk showing v-shaped structures interpreted as shrunken muscle blocks; P, detail of caudal fin with fin rays. Scale bar represents 25 mm (A); 150 μ m (B); 3 mm (C); 6 mm (D–E); 11 mm (F); 24 mm (G); 45 mm (H); 1 mm (I, P); 3.5 mm (J); 4 mm (K); 2.5 mm (L); 0.5 mm (M–N); 1.5 mm (O). *Abbreviations:* EC, Early Life Research Center, Jinning, China; FMNH, Field Museum of Natural History; IGSE, Institute of Geological Science, Edinburgh, UK; NEIU, Northeastern Illinois University; NWU, Department of Geology, Northwest University, Xi'an, China; YKLP (formerly RCCBYU), Yunnan Key Laboratory for Palaeontology, Kunming, Yunnan Province, China.



and they can be accounted for by the elaboration of two embryological phenomena that evolved within stem Olfactores: neurogenic placodes, from which cranial sense organs develop, and neural crest, which is a population of migratory multipotent stem-like cells from which the novel neural, skeletal, pigment cells and their derivative tissues develop (Donoghue *et al.* 2008). The profound elaboration in the fate of neurogenic placodes and neural crest in vertebrates, in comparison with tunicates, has been explained as a consequence of the evolutionary assembly of regulatory interactions among the genes directing development (Nikitina *et al.* 2008). This will have responded to, and may perhaps have been facilitated by, the explosion in coding regulatory genes that occurred as a consequence of one or more whole-genome duplication events (Dehal and Boore 2005), as well as an independent but equally dramatic diversification in noncoding regulatory microRNA diversity, both of which occurred in the vertebrate stem lineage (Heimberg *et al.* 2008, 2010).

While vertebrate innovations are profound, most non-experts would be hard pressed to distinguish the living cyclostomes as vertebrates. For starters, they do not even have vertebrae (although they have rudiments in the form of arcualia; Ota *et al.* 2011). The majority of those features, like vertebrae, that are widely but incorrectly assumed to be general to all vertebrates, were acquired in the gnathostome lineage after it separated from cyclostomes. In some senses, this is an even more dramatic evolutionary episode than the origin of vertebrates more generally, as the inventory of features shared by all living jawed vertebrates, but absent from the ancestral crown vertebrate, is even more extensive. Gnathostome novelties include teeth comprised of dentine, enameloid and cellular bone, a mineralized dermal skeleton comprised of tooth-like scales, mineralized fin radials, paired pectoral and pelvic fins with both an endoskeleton and dermal fin rays, a mineralized braincase, a labyrinth associated with the otic organ that has a horizontal semicircular canal, heterocercal tail, anal fin, differentiated gut, parasymphathetic nervous system, paired external nostrils, externally open endolymphatic ducts, among very many other features. It is not surprising therefore that nonuniformitarian macroevolutionary mechanisms have been invoked to explain the transformation of the plesiomorphic body plan of vertebrates into that of gnathostomes (Ohno 1970).

REFINING VERTEBRATE EVOLUTIONARY HISTORY WITH INSIGHTS FROM THE DEAD

Fossil evidence of the emergence of vertebrates is dominated by gnathostomes with mineralized skeletons, for

obvious reasons. However, the discovery and investigation of Cambrian Burgess Shale type Lagerstätte has yielded the remains of entirely soft-bodied vertebrates, invertebrate chordates, and deuterostomes more generally. These have been recovered principally from the Chengjiang (Hou *et al.* 2004) and Burgess Shale (Briggs *et al.* 1994) Lagerstätte. Cambrian fossils interpreted as members of known lineages include cephalochordates *Cathaymyrus* (Fig. 2E; Shu *et al.* 1996a) and *Pikaia* (Conway Morris and Caron 2012); tunicates *Cheungkongella* (Shu *et al.* 2001a) and *Shankouclava* (Fig. 2C; Chen *et al.* 2003); and vertebrates *Haikouichthys* (Fig. 2K, and its likely junior synonym *Myllokunmingia*; Shu *et al.* 1999, 2003; Hou *et al.* 2002), *Metaspriggina* (Conway Morris 2008; Conway Morris and Caron 2014), *Zhongjianichthys* (Shu 2003) and *Zhongxiniscus* (Luo *et al.* 2001). Fossils described from the Devonian and Carboniferous as hagfishes include *Gilpichthys* (Bardack and Richardson 1977), *Myxineidus* (Poplin *et al.* 2001), and *Myxinikela* (Fig. 2F; Bardack 1991, 1998), and lampreys *Hardistiella* (Janvier and Lund 1983; Lund and Janvier 1986; Janvier *et al.* 2004), *Mayomyzon* (Fig. 2I; Bardack and Zangerl 1968, 1971) and *Priscomyzon* (Gess *et al.* 2006); *Mesomyzon* has been described as a lamprey from the Jurassic Jehol Biota of China (Chang *et al.* 2006). Interpreting the precise phylogenetic position and, therefore, the evolutionary significance of many of these fossils is problematic as the majority of chordate, Olfactores and vertebrate characters are developmental or cytological and therefore not usually fossilized even under conditions of exceptional preservation (Donoghue and Purnell 2005). Phylogenetic resolution is complicated further by the impact of decay on unmineralized vertebrates and chordates, which can affect the loss of anatomical characters in reverse phylogenetic order (Donoghue and Purnell 2009; Sansom *et al.* 2010).

Yunnanozoon and Haikouella

Although the soft-bodied vertebrates described from fossil Lagerstätte are useful in that they provide minimum constraints on the age and palaeobiogeography of the lineages to which they have been attributed, they do not provide much additional insight into the evolutionary origin of vertebrates. This occurs principally because these fossils do not deviate materially from the anatomy of their living representatives and, hence, they have proven comparatively easy to classify. Perhaps more interesting, and concomitantly frustrating, are those soft-bodied fossils that have proven so much more difficult to classify, precisely because they deviate anatomically from living vertebrates and invertebrate chordates. Perhaps, the most interesting of these problemata are *Yunnanozoon* and *Haikouella* (Fig. 2J; which do not differ materially) that have been

interpreted variably as craniates (=vertebrates) with external gills, paired sense organs, a differentiated brain, a notochord against which segmental musculature appears to have been asymmetrically developed and a trunk enveloped in cuticle (Mallatt and Chen 2003). This is not a combination of characters that anyone envisaged in a vertebrate, and therefore, *Yunnanozoon* constitutes a welcome test of hypotheses on the anatomical assembly of the vertebrate body plan. Or perhaps *Yunnanozoon* is such a radical challenge to expectation because interpretations of its anatomy and therefore its systematic classification are incorrect. And, indeed, the interpretations of almost every one of the yunnanozoans' preserved anatomical characters have been contested, and the organisms alternately classified as cephalochordates (Chen *et al.* 1995), stem chordates (Dzik 1995), crown hemichordates (Shu *et al.* 1996*b*, 2004), stem ambulacrarians (Shu 2003), neither chordates nor hemichordates (Bergström 1998), stem deuterostomes (Budd and Jensen 2000; Shu *et al.* 2001*b*, 2004; Shu 2003) and stem bilaterians (Budd and Jensen 2000; Dewel 2000). Whichever, if any, of these systematic interpretations is correct, the yunnanozoans will impact upon our perspective of anatomical character evolution within the deuterostomes. For instance, only vertebrates among deuterostomes have true gills (as opposed to a perforated pharynx; Mallatt *et al.* 2003), yet trunk musculature is radially developed about the notochord in chordates and chordates do not have a cuticularized integument; indeed, cephalochordates lack an integument (Whitewar 1986). However, until the phylogenetic position of the yunnanozoans is resolved, their evolutionary significance will remain moot.

Conodonts

Debate over the systematic position and therefore the evolutionary significance of conodonts is a much longer-running parlour game. Known largely from their microscopic phosphatic tooth-like 'elements', conodonts have been interpreted as fossil plants, algae, all manner of invertebrates and, of course, vertebrates (Müller 1981). The rules of the game were tightened upon discovery of their soft tissue anatomy (Fig. 2L–P), which revealed conodonts to have a notochord (Fig. 2O), segmental trunk musculature (Fig. 2O), a ray-supported midline caudal fin (Fig. 2P), paired sensory organs (Fig. 2M–N) and a short series of gill pouches (Fig. 2N; Briggs *et al.* 1983; Aldridge *et al.* 1986, 1993). Given this suite of anatomical characters, conodonts were readily interpreted as chordates and even vertebrates (Aldridge *et al.* 1993; Donoghue *et al.* 2000), and within this phylogenetic context, the homologies of conodont and vertebrate skeletal tissues considered in more detail. Conodont elements are

bicomponent, comprised of a hypermineralized coarsely crystalline crown tissue that often includes a homogenous vacuolated 'white matter', and a basal tissue that is laminated and or spheritic or includes ramifying tubules. The two tissues are arranged with the crown tissue capping the basal tissue, and incremental growth lines reflect appositional growth. Of all chordate tissues, conodont crown tissue compares best to enamel, and the basal tissue to dentine (Dzik 1986; Sansom *et al.* 1992, 1994; Donoghue 1998). These similarities appear even more significant given that in conodont elements, the crown and basal tissues are arranged topologically and grow in the same manner as enamel and dentine in vertebrate teeth and dermal denticles (Donoghue 1998; Donoghue and Aldridge 2001). Together, these hard and soft tissue homologies have supported a stem gnathostome affinity for conodonts.

However, these putative homologies have been contested, principally in terms of the homology of conodont and vertebrate skeletal tissues (Kemp and Nicoll 1995, 1996; Schultze 1996; Kemp 2002*a, b*; Blicek *et al.* 2010; Turner *et al.* 2010). Ultimately, conodont and vertebrate skeletal tissue homologies have been rejected because it has been demonstrated that the putative homologues were not present in ancestral conodonts, and therefore, they could not have been present in the last common ancestor of conodonts and other vertebrates (Murdock *et al.* 2013*b*). It does not follow, as Blicek, Turner and colleagues have argued (Blicek *et al.* 2010; Turner *et al.* 2010) that conodonts are anything other than vertebrates, an hypothesis that was substantiated explicitly on the nature of soft anatomical characters and not skeletal histological homologies (Donoghue 1998; Donoghue *et al.* 2000; Murdock *et al.* 2013*b*). Indeed, conodonts were revealed to be stem vertebrates by Turner *et al.* (2010). Perhaps more importantly, with a mineralized skeleton that evolved in convergence rather than common descent with gnathostomes, conodonts do not contribute materially to our understanding of the evolutionary assembly of the vertebrate body plan.

Ostracoderms

Thus, for all of the excitement that fossils of soft-bodied vertebrates attract, the fossils that contribute the most to our understanding the evolutionary assembly of the vertebrate body plan are the boney 'ostracoderms' (Figs 1, 3A–G). These are an assemblage of apparently distinct clades that include both jawless and jawed vertebrates, all of which exhibit characters that are exclusive to gnathostomes among the living vertebrates. Chief among these characters is the presence of a mineralized dermal skeleton in which the canonical vertebrate skeletal tissues are



first expressed in evolution, viz. enameloid, dentine and bone. It is this key apomorphy which transforms the fossil record of vertebrates from the exceptional to the routine, recording many subsequent innovations within early vertebrate evolution. Component ostracoderm lineages are related by degree to the living jawed vertebrates, the chondrichthyans and osteichthyans, betrayed by the hierarchically nested suites of characters that they possess (Maisey 1986; Janvier 1996; Donoghue *et al.* 2000).

Thus, for instance, the jawless pteraspidomorphs, including the heterostracans (Fig. 3A), are the first vertebrates with a mineralized dermal skeleton. The anaspids (Fig. 3B) and thelodonts (Fig. 3C) have such a skeleton, but also possess a differentiated gut. Galeaspids (Fig. 3D–E) additionally possess a mineralized cartilaginous neurocranium that reveals details of the gross anatomy of the brain (Fig. 3E); in osteostracans (Fig. 3F), the neurocranium is comprised of perichondral bone, dermal bone is cellular, the tail fin is heterocecral, gill openings are slit shaped, and they possess paired pectoral fins, all features otherwise exclusive to jawed vertebrates. The apparently paraphyletic placoderms (Fig. 3G) record the acquisition of pelvic appendages, mineralized vertebrae and fin radials, a jointed gill skeleton, and also jaws. Thus, the ostracoderms are stem gnathostomes, effectively recording the piecemeal assembly of the body plan shared by all crown gnathostomes (Janvier 2001; Donoghue and Purnell 2005).

THE TIMING AND TEMPO OF EARLY VERTEBRATE EVOLUTION

The timescale of early vertebrate evolution has been interpreted largely in the light of phylogenetically constrained stratigraphy, which reveals extensive ghost lineages in the known record, particularly for stem and, perhaps, crown gnathostomes in the Ordovician (Sansom *et al.* 2001). Furthermore, simple statistical interpretation of stratigraphic occurrence data indicates that these inferences of the missing record are conservative (Donoghue *et al.*

2003). Molecular clocks inevitably infer a Neoproterozoic origin of vertebrates; however, these divergence estimates vary by almost 180 million years (Kumar and Hedges 1998; Blair and Hedges 2005; Erwin *et al.* 2011). The apparent discordance with the vertebrate fossil record may seem unpalatable as, even when interpreted optimistically, the record of crown vertebrates cannot be extended beyond epoch 2 of the Cambrian and, more likely (because of equivocation over the stem versus crown classification of entirely soft-bodied vertebrates), may not be substantiable beyond the Middle Ordovician (Benton *et al.* 2009). Molecular estimates for more exclusive vertebrate clades are in much closer accord, both between divergence time studies, and with the fossil record, an inevitable consequence of the tighter temporal constraints imposed by a fossil record that increases in fidelity with the origin of boney skeletons (cf. Donoghue and Benton 2007). Indeed, it is very likely that the fossil record of early vertebrates provides a poor approximation of their evolutionary history. This inference is underlined by considering the fossil record of conodonts whose mineralized elements occur effectively continuously in all marine facies from Cambrian to Triassic; yet only a handful of fossils preserve their unmineralized anatomy, the oldest of which (Gabbott *et al.* 1995) antedates the first appearance of their skeletal remains by some 60 million years. Fossil calibrations derived exclusively from taxa with poor preservation potential inevitably encompass greater uncertainty, typically described by broad prior probability densities, which provide less precise posterior age estimates. Intriguingly, Erwin *et al.* (2011) provided consistently younger divergence estimates for the major vertebrate nodes. This study encompasses a broad taxon sample of metazoan clades, some of which have a Precambrian fossil record, which effect to better constrain the age of deeper nodes within the tree and therefore better inform estimates of the age of vertebrate lineages, predicting a Late Ediacaran origin of crown Olfactores, a Middle Cambrian radiation of crown vertebrates and an earliest Devonian divergence of crown osteichthyans (Erwin *et al.* 2011).

FIG. 3. Jawless and jawed ostracoderms and crown gnathostomes. A, *Errivaspis waynensis* (NHMUK P19789), an pteraspidid heterostracran from the Lochkovian (early Devonian) of Herefordshire, England. B, *Birkenia elegans* (NHMUK P10141), an anaspid from the Llandovery (early Silurian) of Scotland. C, *Loganellia scotica* (GLAHMV8304), a thelodont from the early Silurian of Lesmahagow, Scotland. D–E, headshields of the galeaspids *Nochelaspis maeandrine* (IVPP V10106-1) (D) and *Duyunolepis paoyangensis* (GMC V1324) (E) from the early Devonian of China, the latter preserving a natural endocast of an originally mineralized braincase (reproduced with permission from Liu *et al.* in press). F, *Zenaspis waynensis* (NHMUK P24940), an osteostracran from the early Devonian of Herefordshire, England, preserved in dorsal aspect and preserving the dextral pectoral fin tucked between the conular process and the trunk; G, *Ctenurella gladbachensis* (MB.f.5533), a pycnodont ‘placoderm’ from the late Devonian of Germany; H, *Ptomacanthus anglicus* (NHMUK P19998), an early Devonian ‘acanthodian’ from Herefordshire, England, which has recently been assigned to both the gnathostome stem (Davis *et al.* 2012) and crown (Brazeau 2009; Zhu *et al.* 2013). Scale bar represents 12 mm (A); 6 mm (B); 11 mm (C); 25.5 mm (D); 16 mm (E); 17.5 mm (F); 19.5 mm (G); 29 mm (H). *Abbreviations:* GLAHM, Hunterian Museum, Glasgow, UK; GMC, Geological Museum of China (Beijing); IVPP, Institute of Vertebrate Palaeontology and Palaeoanthropology, Beijing, China; MB, Museum für Naturkunde in Berlin; NHMUK, Natural History Museum, London, UK.

While there are some constraints on the timescale of early vertebrate evolution, our understanding of its tempo, in terms of the rate of phenotypic character evolution, remains crude. The genome of almost all living vertebrates preserves the phylogenetic footprint of at least two rounds of whole-genome duplication, once in the vertebrate stem lineage and again in the gnathostome stem lineage (or perhaps just twice in the vertebrate stem lineage); additionally, a third broadly inherited whole-genome duplication event occurred in the teleost stem lineage (Donoghue and Purnell 2005; Kuraku *et al.* 2009; Smith *et al.* 2013). The impact of these events upon phenotypic evolution is not clear; it has been argued that these events facilitated developmental and phenotypic evolution deterministically (Wagner *et al.* 2003), or at least permissively (Holland 1992); yet it remains possible that the phylogenetic footprint of genome duplication remains so clear because the gene regulatory controls on development were already sufficiently complex that their functions could be partitioned among duplicated genes, preventing their loss through drift (Donoghue and Purnell 2005). In this case, the fossil record indicates that in the assembly of the gnathostome bauplan, there was nothing unusual about the tempo and mode of phenotypic character evolution, as might be expected by the hypothesis of whole-genome duplication driving developmental evolution (Donoghue and Purnell 2005), although this may merely reflect the possibility that the first two whole-genome duplication events occurred within the vertebrate, and not the gnathostome, stem lineage (Kuraku *et al.* 2009; Smith *et al.* 2013). Thus, the evolutionary assembly of the gnathostome solution may be a permissive consequence of these events. Better constraints on genome evolution among chordates, provided by better assemblies of cyclostome genomes, are key to resolving this conundrum concerning the relationship between the evolution of phenotype and genotype.

WHAT REMAINS?

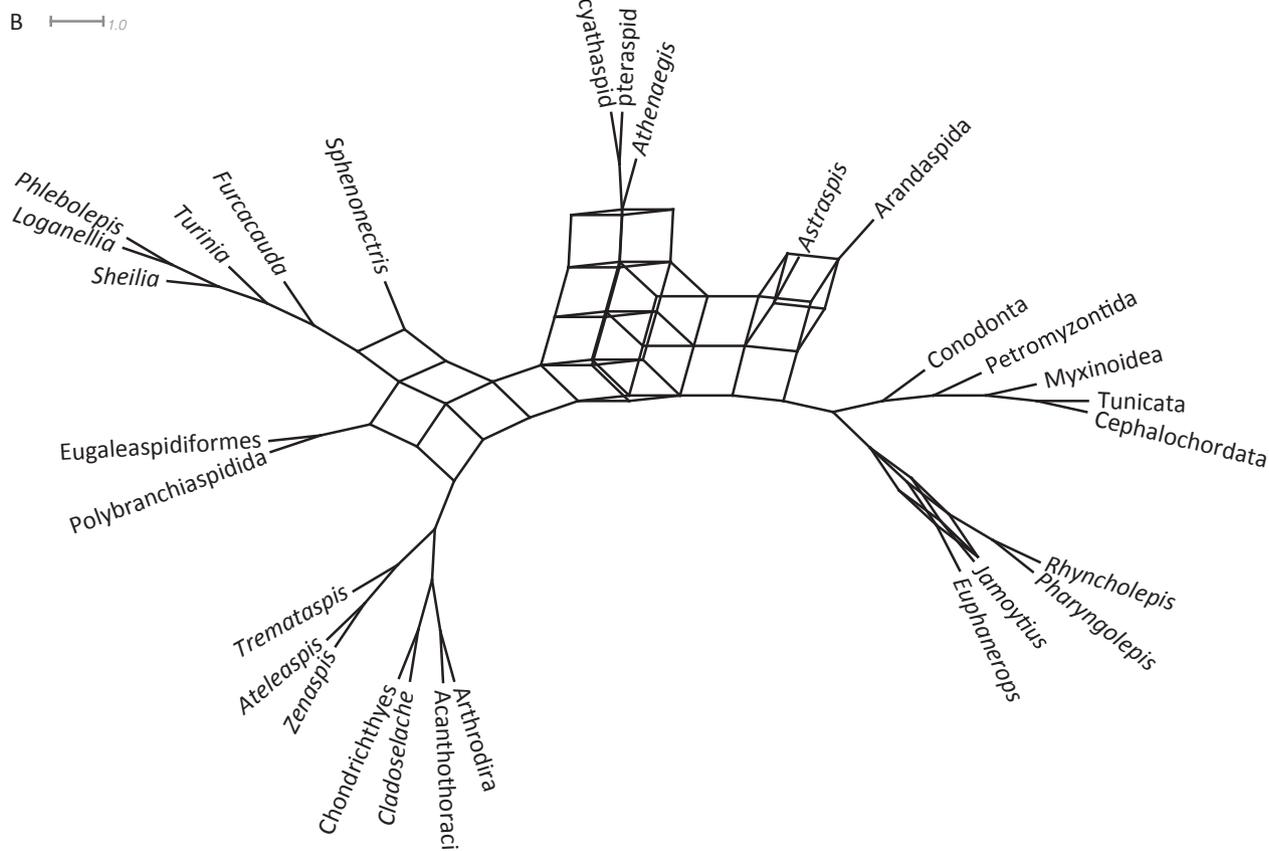
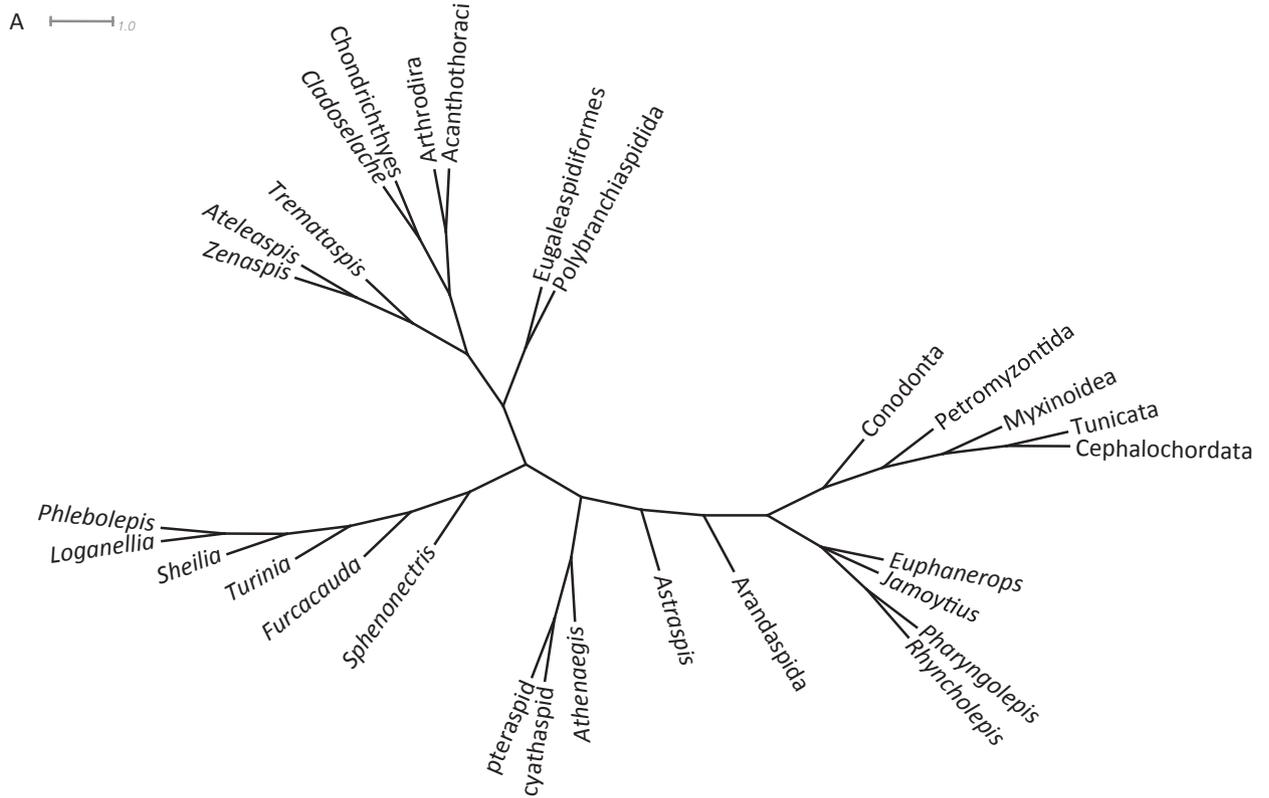
It should go without saying that our knowledge of early vertebrate evolution relies not merely upon the anatomical interpretation of its fossil record but upon our understanding of the phylogenetic relationships among the fossil species, and among their living relatives. Early vertebrates were long an exemplar of phylogenetic rigour in animal systematics; indeed, they constituted the very battleground on which competing approaches to phylogenetic analysis were debated (Janvier 1979; Halstead 1982).

However, the scale of such phylogenetic studies has not extended much beyond these earliest incarnations, invariably considering either the internal relationships of component plesions or else the interrelationships among exemplars of plesions. Early vertebrates have yet to be subjected to rigorous tests of the monophyly of the plesions of stem gnathostomes recognized traditionally, which are constructs based on similarity. It is likely that these encompass both shared derived from shared primitive similarities.

Tests of the coherence of traditional plesions of jawed vertebrates have found placoderms and acanthodians (Fig. 3H) wanting, now recognized as a series of successive sister lineages of stem gnathostomes and stem chondrichthyans, respectively (Brazeau 2009; Davis *et al.* 2012; Zhu *et al.* 2013; Dupret *et al.* 2014). The putative clades of jawless stem gnathostomes have undergone no such revision and, indeed, where their monophyly has been tested, clades such as the thelodonts and anaspids have been found to be paraphyletic (Donoghue and Smith 2001; Blom 2012). The scale of the problem can, perhaps, be evaluated through a consensus network analysis of the trees derived from the study by Donoghue and Smith (2001).

Consensus networks are a type of consensus tree that summarizes not only the common topologies present in the source trees (at a specified threshold level of occurrence), but also the conflicting topologies indicated by the data (Moulton and Huber 2009). Like regular cladograms, in consensus networks, the internal branches reflect relationships among the taxa and the nodes reflect possible common ancestors, and the branch weights reflect the number of input trees in which a given split is manifest (Moulton and Huber 2009). However, the consensus network also represents conflict in the data by also presenting conflicting relationships among the taxa. The more strictly bifurcating the cladogram, the less conflict present in the data. Donoghue and Smith (2001) recovered 37 most parsimonious trees through parsimony analysis of a data set that encompassed multiple representatives for each of the stem gnathostome plesions. A consensus network for splits with a weight threshold of 0.5 (i.e. including relationships supported by half or more of the source trees) is well resolved (Fig. 4A). However, when lower weight thresholds are considered, the strictly bifurcating nature of the consensus network breaks down, yielding increasingly net-like consensus trees at weight thresholds of 0.25 and 0.10 (Fig. 4B). These indicate that, given the data, there is robust support for the relationships among living invertebrate chordates and vertebrates,

FIG. 4. Consensus networks calculated from the 37 most parsimonious trees derived from analysis of the data from Donoghue and Smith (2001). A, splits included above a weight threshold of 0.50. B, splits included above a weight threshold of 0.10. In both cases, edge lengths reflect weight, that is percentage of most parsimonious trees (mpts) which recover any given edge.



for the monophyly and successive branching order of conodonts, anaspids, osteostracans and jawed vertebrates, but the relative relationships among heterostracans, thelodonts and galeaspids are less clear. Given the artificiality of the phena (higher taxa), the volume of missing data and the poor overlap in the classes of characters that can be coded for between living and fossil chordates (including vertebrates; Donoghue *et al.* 2000), the degree of conflict in the phylogenetic relationships suggested by the data is concerning.

A serious attempt to test the monophyly of the stem gnathostome plesions could build on recent analyses to establish ingroup relationships within the Anaspida (Blom and Märss 2010; Blom 2012), Galeaspida (Zhu and Gai 2006), Heterostraci (Pernègre and Elliott 2008; Lundgren and Blom 2013) and Osteostraci (Sansom 2009). However, there is a limit to the number of characters that can be extracted from the skeletons of these fossil fishes, particularly those that are currently resolved as branching early within the gnathostome stem, viz. anaspids, heterostracans and thelodonts, fossils of which comprise little more than scale-studded silhouettes. These data are not yet exhausted. There is little depth or breadth to our knowledge of dermoskeletal histology in any of the stem gnathostome plesions, and the existing data for groups such as the anaspids (Gross 1938, 1958; Blom *et al.* 2002) and galeaspids (Janvier 1990; Zhu and Janvier 1998; Wang *et al.* 2005) are very poorly understood. The external cranial anatomy of galeaspids has been well characterized, the detailed investigation of endocranial anatomy has but begun (Gai *et al.* 2011), and postcranial anatomy is virtually unknown. The pituriaspids, an apparently distinct plesion of stem gnathostomes, are known only from a small number of external and partial internal moulds (Young 1991). Nevertheless, it is likely that the core of the putative plesions of stem gnathostomes will be corroborated as clades, leaving a residue of lineages and taxa that remain phylogenetically unresolved for lack of data.

There is great room for improvement in our knowledge of the timing and tempo of early vertebrate evolution. The thorough phylogenetic review that we propose will serve as a foundation for a revised timescale that is based not merely on phylogenetically constrained stratigraphic occurrence but, rather, upon molecular divergence time estimates integrating both morphological and molecular data, and both fossil and living taxa (Pyron 2011; Ronquist *et al.* 2012). In this way, it is possible to date not merely the divergences among living and fossil species but, consequently, the rate of phenotypic character evolution and of diversification, facilitating more effective tests of the impact of whole-genome duplication in effecting the assembly of the vertebrate and gnathostome body plans and their ensuing taxic and ecological diversification.

The ecology of early vertebrates has been the topic of much speculation, based both on living and fossil species. For instance, the 'New Head Hypothesis' proposes that early vertebrate evolution is characterized by a trend towards increasingly active food acquisition (Gans and Northcutt 1983; Northcutt and Gans 1983). Yet the ecology of early vertebrates has been the subject of pitifully little rigorous attention (Purnell 2001). Conodonts are by far the best understood in terms of ecology, from functional analyses of their feeding elements using microwear, microstructural, occlusal kinematic and finite elements analyses (Purnell 1995; Donoghue and Purnell 1999; Donoghue 2001; Jones *et al.* 2012*a, b*; Purnell and Jones 2012; Murdock *et al.* 2013*a*; Martínez-Pérez *et al.* 2014), although they have been applied to few conodont species. Nevertheless, they establish methodological and experimental approaches that can be employed in studies of feeding ecology in other groups of early vertebrates, particularly the stem and crown gnathostomes. Indeed, microwear has been employed to test among hypotheses of micro- versus macrophagy in heterostracans (Purnell 2002); there is scope for further work on heterostracomorphs, and this approach could be extended readily to osteostracans, placoderms and acanthodians whose feeding plates are not uncommonly preserved. Similarly, disparity analyses of mandible characteristics have been employed to characterize the expansion in feeding ecologies during the initial radiation of jawed vertebrates (Anderson *et al.* 2011). Speculative hypotheses of feeding function in these and other groups, including galeaspids, thelodonts and anaspids, can be tested using computed fluid dynamics (Shiino *et al.* 2009). There is certainly no shortage of hypotheses in circulation (Purnell 2001), and the shift of focus from speculation based on vague analogy, to quantitative tests of these hypotheses, is long overdue.

Finally, although we raise serious concern about the validity of traditional groups of early vertebrates, stem gnathostomes most especially, the backbone of the relationships among heterostracans, galeaspids, osteostracans and jawed vertebrates will surely withstand all scrutiny because it is founded on the nested hierarchy of characters that evidence the evolutionary assembly of the gnathostome body plan. It is concerning, however, that although the fossil record evidences the acquisition of the fundamental characters that distinguish living jawed from jawless vertebrates, it, or our understanding of it, is so far silent on the evolutionary origin of jaws themselves. Illumination may yet be found through further anatomical and phylogenetic scrutiny of the fossils that occupy museum drawers, but the known facies-driven gaps in the fossil record of early vertebrate evolution along with phylogenetic–biogeographic inconsistencies (Smith *et al.* 2002; Blicek and Turner 2003), and the discovery of cryp-

tic taxa like the pituriaspids, all combine to suggest that fundamental insights into this evolutionary episode reside in rock, awaiting discovery.

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