

# The origin and early evolution of chordates: molecular clocks and the fossil record

*Philip C.J. Donoghue, M. Paul Smith and Ivan J. Sansom*

---

## ABSTRACT

Evolutionary biology abounds with theories and scenarios for the origins of the major chordate clades but little attempt has been made to constrain knowledge over the dating of these evolutionary events. The fossil record of early chordates, including stem-gnathostomes and basal crown-gnathostomes, as well as the sister-clade Ambulacraria (Hemichordata plus Echinodermata), is critically re-evaluated. This is achieved through both qualitative and quantitative assessment of the fit of phylogenetic hypotheses to stratigraphic range data, and through assessment of the internal consistency of stratigraphic range data. The results suggest that the fossil record of early chordates is of variable quality; the fossil record of basal chordates appears to be a poor reflection of their evolutionary history, while the fossil record of many stem-gnathostomes, such as conodonts and heterostracans, appears to be very good, albeit poorly understood in places. Thus, palaeontological data provide little constraint on the origin of chordates, craniates, and vertebrates, other than to indicate that these clades were established by 530 Ma. The origin of total-group Gnathostomata has a well-supported fossil estimate of 495 Ma, a date which falls within the error calculations of published molecular clock estimates. The origin of crown-gnathostomes is dated at 457 Ma using the fossil record, with a confidence interval extending to 463 Ma, implying an incomplete record; this lies just outside molecular estimates (e.g. 528 Ma  $\pm$  56.4 myr). Finally, the fossil record suggests the divergence of actinopterygians and sarcopterygians at 425 Ma, with a very narrow confidence interval (+ 580 Ka) and falls within molecular estimates (450 Ma  $\pm$  35.5 myr). Thus, where internal assessments of palaeontological data imply a good record there is correlation with molecular clock estimates, and where these assessments suggest a poor record there is poor correlation. Where correlation occurs we may assume that our estimates are a good reflection of the true time of divergence of the various clades, and where there is conflict we must assume nothing. We note that even where corroboration between datasets occurs, error bars on divergence times remain too coarse to attempt correlation to evolutionary events in other clades, and extrinsic events in Earth history.

## The problem

It is of course anthropocentric bias, but the nature of the evolutionary and environmental events surrounding the origin and early evolution of the phylum Chordata

are some of the most extensively researched problems in evolutionary biology. Theories that have sought to account for these events are contingent upon shifts in calibration of the geological timescale, a fossil record that is dynamic both in terms of new discoveries and reinterpretation of the phylogenetic affinities of old finds and, more recently, the introduction of molecular clock estimates for the times of divergence of living clades. It is therefore not surprising that many such hypotheses have fallen purely because events once thought to be coeval are revealed not to be so. But with so many lines of evidence, many of which are independent, there remains the possibility that conflict may give way to conciliation, rather than merely to compromise. Recent advances have resulted in a considerable fleshing out of the early fossil record of chordates (Sansom *et al.* 2001; M.P. Smith *et al.* 2001, 2002), the geological timescale is now more finely calibrated than at any time in the past (e.g. Remane 2000), and there is an ever increasing database of molecular sequences for analysing evolutionary relationships and sampling for molecular clock analyses. With these developments, understanding the events surrounding the origin and early evolution of the chordate phylum may now prove more tractable than at any time previously.

### The data

The nearest living relatives of the chordates are the echinoderms and hemichordates and, together, these three phyla comprise the Deuterostomia. Living invertebrate chordates are a very depauperate group in comparison with their vertebrate relatives, comprising two or three groups depending upon how the Vertebrata are defined. The most plesiomorphic groups are the tunicates and cephalochordates, and although there has historically been a great deal of prevarication surrounding their interrelationships, the tunicates are now widely recognized as the most basal group of living chordates. The next most inclusive clade, Craniata, includes only the hagfishes in addition to the vertebrates, which are in turn composed of the lampreys plus the Gnathostomata (living jawed vertebrates). Gnathostomes, in turn, are composed of chondrichthyans, and the two most derived groups, the actinopterygians and sarcopterygians, which includes the lineage leading to tetrapods.

These taxonomic groups are defined solely on the basis of living taxa, and so it is possible to provide molecular estimates for the divergence of the various groups without recourse to the fossil record for anything other than internal and/or external calibration. However, the divergence of the various lineages does not equate to the origin of the taxonomic groups, at least not in the sense that most biologists understand these taxa. This is because most of these groups also include fossil taxa, with varying degrees of taxonomic diversity and disparity, which are part of the lineage leading to the crown-group of living taxa, but do not possess the full suite of anatomical characteristics necessary for inclusion within the crown-group (Jefferies 1979). For instance, the extinct osteostracans are a group of jawless vertebrates that share a number of derived characters with gnathostomes that they do not share with lampreys. Hence, osteostracans are resolved as more closely related to gnathostomes than lampreys and represent part of the lineage leading to gnathostomes after its divergence from that last common ancestor shared with lampreys. However, since they lack jaws, osteostracans cannot be considered part of the group 'Gnathostomata' as perceived by most biologists. Current hypotheses of early vertebrate relationships

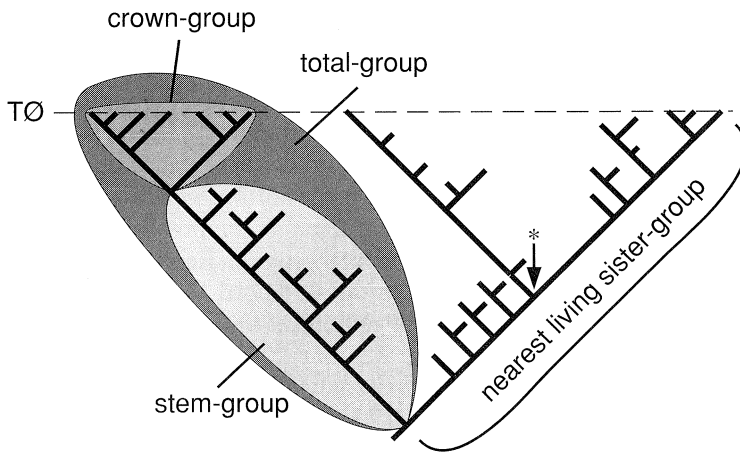


Figure 10.1 The relationships between stem-, crown-, and total-groups.  $T_0$  is time zero (present day). \* denotes the time of origin of a crown-group and its two constituent total-groups. After Jeffries (1986).

indicate that there are many such groups that are more closely related to gnathostomes than lampreys. Systematists have devised a means of obviating this state of taxonomic purgatory by allying such lineages with their nearest living group to form an inclusive 'total-group' version of the existing taxonomic concept (Figure 10.1). Thus, the extinct members of the gnathostome lineage that fall outside of crown-group Gnathostomata become part of the total-group Gnathostomata, composed of the paraphyletic 'stem'-lineage leading to a 'crown'-group circumscribed by the living representatives of the Gnathostomata, and including all of their descendants, fossil and extant. The difference between the time of origin of the total-group and that of the crown-group (approximately equivalent to the original concept 'Gnathostomata') reflects the time gap between the point of divergence from the lamprey lineage, and the origin of the living clade, palaeontological estimates for the timing of which differ by as much as 60 million years. Both events are approachable through molecular clock theory (because the time of origin of one crown group is also the time of origin of its two constituent total groups (see figure 10.1)), although they are often confused and/or conflated, and it is usually only the origin of the total-group that is calculated owing to the ease of data collection and the reduced reliance upon subsidiary hypotheses of intrarelations of constituent crown-group taxa.

### **The fossil record of chordates and their near relatives**

#### **Echinoderms**

The earliest putative echinoderms are both Neoproterozoic in age. Subsequent to its description, *Tribrachidium* (Glaessner and Wade 1966) has generally been excluded from contention as an echinoderm (e.g. Wills and Sepkoski 1993), but debate concerning the affinities of *Arkarua* (Gehling 1987) continues. Budd and Jensen (2000) have argued that data in support of echinoderm affinity for the latter taxon are

tenuous and limited exclusively to the presence of pentamerous symmetry. More recent interpretations of echinoderm skeletal homologies following the extraxial–axial theory identify many more echinoderm synapomorphies and symplesiomorphies in the still poorly known anatomy of *Arkarua*. These include a body wall dominated by extraxial rather than axial components, upwardly-oriented perforate extraxial and axial rays, flooring plates that follow the ocular plate rule, and a disc-shaped morphology akin to the edrioasteroids (David and Mooi 1998; Mooi and David 1997, 1998; Mooi 2001; Mooi pers. comm. 2002). The logical extension of this argument is that *Arkarua* is the sister-taxon to all other echinoderms, representing the only member of the echinoderm total-group to lack a stereom skeleton plesiomorphically. However, it should be remembered that all identified homologies are contingent upon the *a priori* assumption of an echinoderm affinity for *Arkarua* and alternative phylogenetic frameworks would lead to a very different interpretation of homologies. Thus, although we will discuss the implications of a Neoproterozoic echinoderm record for the evolutionary history of chordates, this record should not be considered beyond reproach.

Echinoderms are well represented amongst Early Cambrian faunas (see e.g. Smith 1988a,b, 1990), but the precise affinity of these taxa remains the subject of wide-ranging debate. The helicoplacoids are generally considered stem-group echinoderms, slightly more derived than *Arkarua*, but the phylogenetic position of *Camptostroma*, the edrioasteroids, and the carpoids remains contentious, with some authors placing them in stem-echinoderm positions, and others resolving them as members of the echinoderm crown-group; see Smith (1984, 1988a,b, 1990), Sumrall (1997) and David and Mooi (1999; Mooi and David 1998; David *et al.* 2000) for the different arguments, and Mooi (2001) for a compilation of trees reflecting the different hypotheses.

### Hemichordates

The early fossil record of hemichordates is limited in large part to the pterobranchs and extends back to the Middle Cambrian (Bengtson and Urbanek 1986; Durman and Sennikov 1993); the record of enteropneusts does not extend beyond the Jurassic (Arduini *et al.* 1981). These data are widely accepted, but the earliest possible record is of *Yunnanozoon* from the Lower Cambrian Chengjiang Lagerstätten of China. The original and most valid interpretation of this organism to date is as a metazoan of unknown affinity (Hou *et al.* 1991). *Yunnanozoon* has subsequently been described both as a chordate (Chen *et al.* 1995; Dzik 1995) and as a hemichordate (Shu *et al.* 1996a), whilst the suspiciously similar *Haikouella* has also been described as a craniate (Chen *et al.* 1999). The mélange of characters exhibited by *Yunnanozoon* (e.g. Dzik 1995) may indicate a more appropriate placement in the deuterostome stem-group.

### Chordates

Fossil representatives have been claimed for all living groups of invertebrate chordates and jawless vertebrates as far back as the Early Cambrian. Putative fossil tunicates include *Cheungkongella* (Shu *et al.* 2001a), *Palaeobotryllus* (Müller 1977), and *Peltocystis* (Jefferies *et al.* 1996). Putative acraniate chordates include *Lagynocystis* (Jefferies 1973), *Pikaia* (Conway Morris 1979), *Yunnanozoon* (Chen *et al.* 1995;

Dzik 1995), and *Cathaymyrus* (Shu *et al.* 1996b). Possible fossil representatives or close relatives of the living jawless vertebrates include the Cambrian taxa *Haikouella* (Chen *et al.* 1999; but see above), *Myllokunmingia* and *Haikouichthys* (Shu *et al.* 1999), and the Carboniferous taxa *Gilpichthys* and *Pipiscius* (Bardack and Richardson 1977), *Mayomyzon* (Bardack and Zangerl 1968, 1971), *Myxinikela* (Bardack 1991, 1998), and *Hardistiella* (Janvier and Lund 1983; Lund and Janvier 1986), as well as a number of mitrates such as *Mitrocystites* (Jefferies 1967) and *Placocystites* (Jefferies and Lewis 1978).

In addition, there are a wide variety of fossil jawless vertebrates characterized by an extensively developed dermal 'armour' and historically grouped together as the 'ostracoderms'. These include the anaspids, galeaspids, heterostracans, osteostracans, and thelodonts (see Janvier 1996b for an introduction to these various groups). Amongst the jawed vertebrates, there are also a number of large groups that have no living representatives, principally including the placoderms and acanthodians. There is also a swathe of basal chondrichthyans, actinopterygians, and sarcopterygians that belie the apparent disparity of their living relatives.

### **The phylogenetic relationships of living and extinct chordates and their near relatives**

To compare palaeontological and molecular estimates for the time of divergence of the various chordate clades it is first necessary to resolve the phylogenetic relationships of the living and fossil groups of chordates and their near relatives; palaeontological estimates can then be provided through calibration of the resulting phylogeny to the stratigraphic occurrence of the various groups within the geological timescale.

#### ***The calcichordate–stylophoran problem***

No discussion of early chordate evolution would be complete without a consideration of the 'calcichordates'. Jefferies (1967 *et seq*) identifies an extinct group of calcite-plated invertebrates, otherwise interpreted as basal echinoderms (Stylophora; e.g. Ubags 1968; Paul and Smith 1984), as paraphyletic suites of lineages that interleave the stems of extant echinoderms, cephalochordates, tunicates, and vertebrates. This theory has been criticized on many grounds. Amongst the most substantive of these, independent phylogenetic analyses resolve tunicates as basal chordates (García-Fernández and Holland 1994) rather than as the sister-group to the vertebrates, which is a requirement of the 'calcichordate' hypothesis (Jefferies 1986). Furthermore, independent phylogenetic analyses (Peterson 1995) recognize that the cornute and mitrate 'calcichordates' share a number of potential homologies that may only be rejected by weighting other characters that are deemed on the basis of the calcichordate theory to be of greater phylogenetic significance (Ruta 1999). This appears to preclude not only the calcichordate theory, but also Gee's compromise hypothesis that the 'calcichordates' are a paraphyletic ensemble of basal deuterostomes, some of which are more closely related to one or more phyla, than are others (Gee 2001; although it does not preclude the possibility that they are basal deuterostomes). Thus, the stylophorans are not germane to understanding the timing of chordate diversification and we will not discuss them further.

### **Morphological analysis**

The interrelationships of both living and fossil chordates have been the subject of controversy since the origin of systematic classification. Much debate has centred on the relative relationships of the living jawless vertebrates, the hagfishes and lampreys, to living jawed vertebrates, and the implications that this has for the interrelationships of extinct groups of jawless vertebrates and invertebrate chordates. All three possible solutions to the problem of hagfish–lamprey–jawed vertebrate interrelationships have been proposed, but of these, cyclostome monophyly ((hagfish, lamprey) jawed vertebrate) and cyclostome paraphyly (hagfish (lamprey, jawed vertebrate)) have received by far the most attention. Although morphological data were formerly interpreted to support cyclostome monophyly (e.g. Stensiö 1927, 1968; Yalden 1985), the application of phylogenetic systematics to the same dataset led to a revised interpretation of cyclostome paraphyly (Løvtrup 1977; Janvier 1996a, 1981; Hardisty 1982; Forey 1984), a view that is still defended by morphologists (e.g. Janvier 1998; Donoghue *et al.* 2000; Donoghue and Smith 2001). We will consider the implications of both hypotheses in assessing the completeness of the chordate fossil record.

The hypothesis of relationships that we have adopted to provide palaeontological estimates of divergence times for the various chordate clades is a development of the analysis undertaken by Donoghue *et al.* (2000), to include the recently discovered groups of invertebrate chordates and basal vertebrates from the Lower Cambrian Chengjiang Lagerstätte. The results of this extended analysis are presented in Figure 10.2 and the codings for additional taxa are included in Appendix 10.1.

### **Molecular analysis**

In contrast to morphological datasets, analyses of molecular datasets universally resolve the living jawless vertebrates as monophyletic. Although phylogenetic analysis of incomplete mitochondrial datasets resolved hagfishes and lampreys as paraphyletic (Suzuki *et al.* 1995), analysis of the entire mitochondrial genome provides unequivocal support for the monophyly of hagfishes and lampreys (Delarbre *et al.* 2002). Similarly, small datasets of nuclear DNA have provided support for the paraphyly of the living jawless vertebrates (Suzuki *et al.* 1995), but larger datasets provide strong support for monophyly (Goodman *et al.* 1987; Kuraku *et al.* 1999; Hedges 2001). Analysis of RNA also strongly supports cyclostome monophyly (Stock and Whitt 1992; Mallatt and Sullivan 1998; Mallatt *et al.* 2001), although analysis of RNA datasets partitioned into small- and large-subunit components provides conflicting support for both hypotheses (Zrzavý *et al.* 1998).

Resolution of the interrelationships of hagfishes and lampreys is critical to understanding character evolution at the origin of vertebrates and gnathostomes. However, both groups have a comparable fossil record, and there are no known intermediate taxa with a fundamentally earlier or later first appearance in the fossil record than sister and ingroup clades (the reality is quite the opposite). Thus, the difference between the two most likely resolutions of hagfish–lamprey–jawed vertebrate interrelationships is not critical to our understanding of the timing of early chordate diversification or the relationship between the fossil record and molecular clocks.

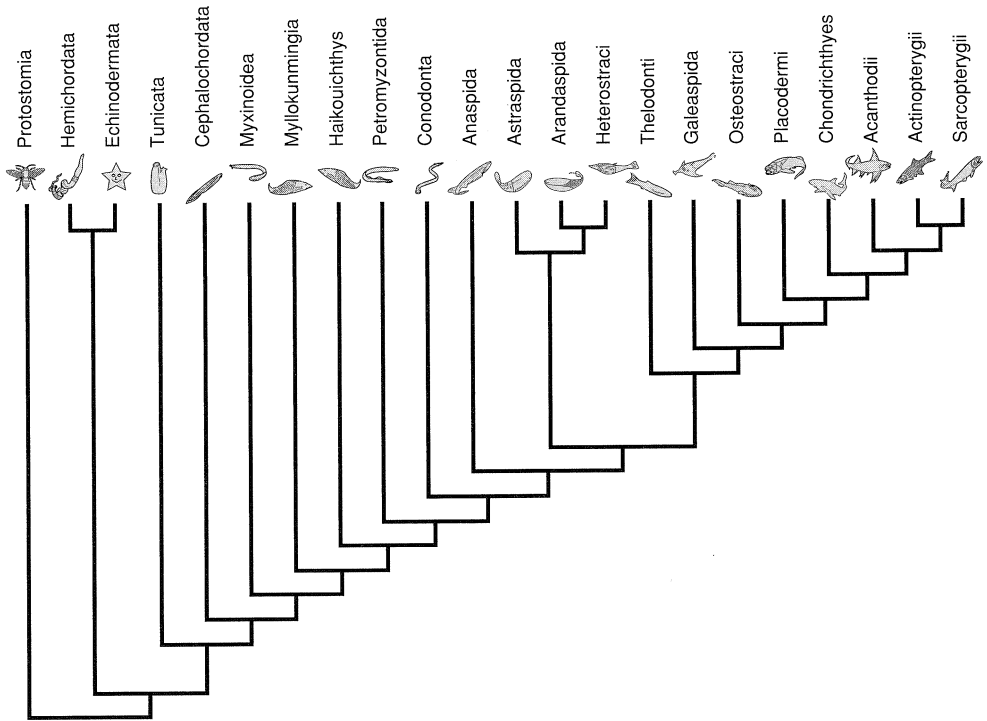


Figure 10.2 The interrelationships of living and extinct groups of chordates and their nearest living relatives.

### Stratigraphic analysis

Although stratigraphic range data are often taken at face value in attempts to calibrate molecular clocks and phylogenetic trees, and to provide palaeontological estimates for the divergence times, various techniques exist to provide confidence limits on the first and/or last appearance of taxa based upon the quality of the intervening record. The chief method for this is gap analysis which was originally developed to provide confidence limits on stratigraphic range data in local sections (Marshall 1990), but can be (Marshall 1990) and has been (Bleiweiss 1998) applied to global datasets provided that the distribution of fossil-bearing horizons within the observed range is random. The technique provides a means of determining limits of probability on how far outside the known stratigraphic range of a taxon the true first (and/or last) appearance might occur – this is proportional to the density with which the taxon has been found throughout its known stratigraphic range (Marshall 1990). It follows that the greater the number of horizons from which the taxon has been recorded, the less likely it is that the true range lies far beyond the limits of the known range, and *vice versa*. Gap analysis calculates, at a given level of confidence (e.g. 95 or 99 per cent), an interval within which the true end point (appearance or disappearance) of a stratigraphic range lies (Marshall 1990). At its simplest, the calculation assumes constant fossil recovery potential, but techniques have been developed to

Table 10.1 Confidence intervals calculated on the basis of the internal relationships of the plesions included in the main analysis.

Taxon	Base	Top	n	Sil P > 0.95	Sil P > 0.99	Ord n	Ord P > 0.95	Ord P > 0.99
<sup>a</sup> Myxinoidea	304	0	2	6080	30 400			
<sup>a</sup> Petromyzontida	325	0	2	6500	32 500			
Conodonts	495	418	670	495.345573	495.531871	501	495.312491	495.48115
Arandaspida	477	464	5	491.491653	505.10961			
Astraspis	457	453	36	457.357449	457.5625			
Heterostraci	428	418	110	428.278649	428.431545			
Anaspids	433	418	57	433.824279	434.285667			
Thelodonts	457	418	295	457.399425	457.615699	7	466.065686	466.065686
Galeaspids	438	418	9	447.084309	453.565588			
Osteostracans	433	418	25	434.994205	436.172915			
Placoderms	428	418	11	431.492828	433.848932			
Chondrichthyans	457	418	67	458.810995	459.818422	9	463.359016	463.359016
Acanthodians	446	418	178	446.477934	446.738061	1		
Actinopterygians	425	418	59	425.371054	425.578457			
Sarcopterygians	423	418	1					

'Sil n' is the number of records within the interval Cambrian–Silurian, and 'Sil P > 0.95' and 'Sil P > 0.99' are 95 and 99 per cent confidence intervals on the first appearance of the plesion respectively, based upon the Cambrian–Silurian interval. 'Ord n' is the number of records within the interval Cambrian–Ordovician, and 'Ord P > 0.95' and 'Ord P > 0.99' are 95 and 99 per cent confidence intervals on the first appearance of the plesion, respectively, based upon the Cambrian–Ordovician interval.

<sup>a</sup> The fossil record of hagfishes and lampreys is limited to the Carboniferous and, as a result, the confidence interval calculations are based on their full stratigraphic range, rather than limited to the pre-Devonian as are the other calculations.

incorporate variable recovery potential that may result, for example, from biases in facies preservation arising from changes in relative sea level (Holland 1995, 2000; Marshall 1997; Tavaré *et al.* 2002).

We have calculated 95 and 99 per cent confidence limits for the fossil record of each of the main groups of fossil and living invertebrate chordates and jawless and jawed vertebrates using the combined micro- and macrofossil record. The values are presented in Table 10.1 and are graphically expressed in Figure 10.3.

## Assessing congruence between cladograms and stratigraphy

### Ghost lineages and their conceptual efficacy

Following the principle that sister taxa are derived from common ancestors and, thus, have an evolutionary history that can be traced back to the point in time at which they diverged from their latest common ancestor, an assessment of the completeness of the fossil record of a taxon can be achieved through comparing the stratigraphic ranges of sister taxa. The inferred range extension of a taxon based on the longevity of its sister taxon is known as a 'ghost lineage' or 'ghost range', a concept introduced by Gauthier *et al.* (1988) and developed by Norell (1992) amongst others. The technique is useful because it provides a means of inferring the existence of unsampled or unsampleable taxa, but it relies upon a number of important and potentially limiting assumptions. First, it must be assumed that the cladogram is a faithful reflection of evolutionary relationships. Second, all the taxa in the cladogram must be



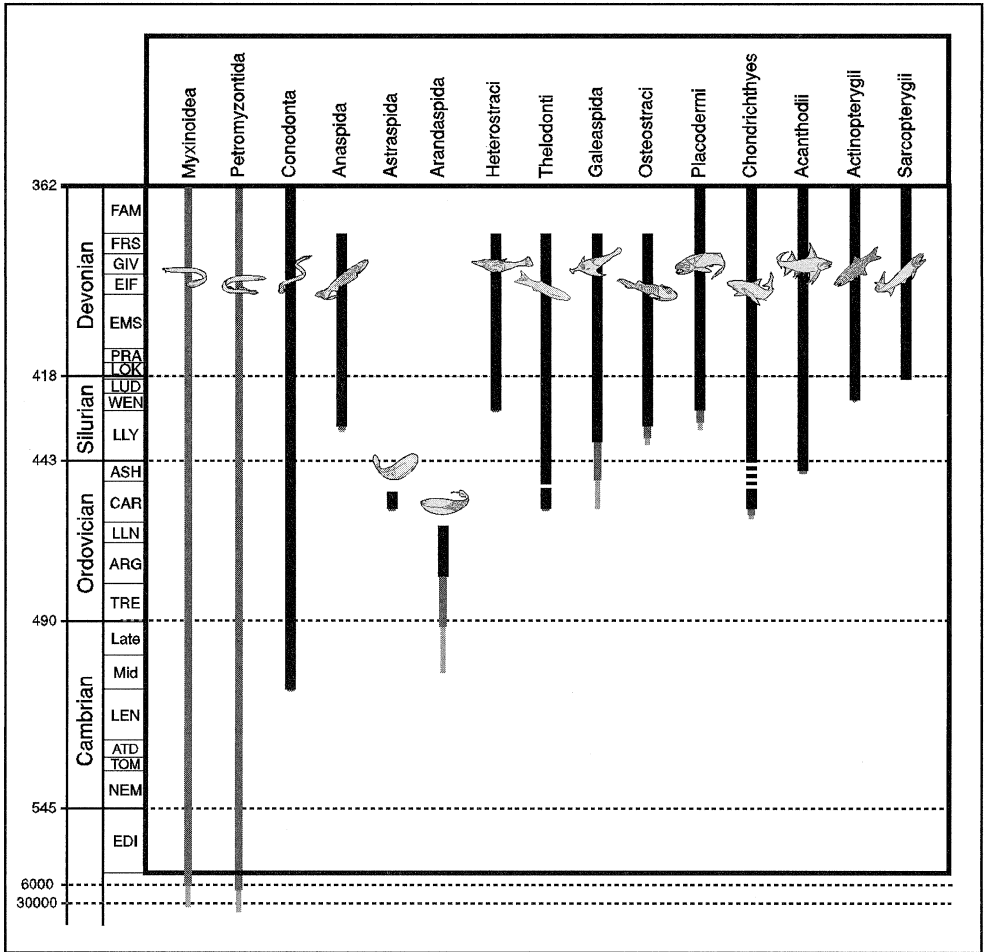


Figure 10.3 Stratigraphic range data for the major extinct and extant groups of vertebrates. Observed stratigraphic range represented by thick black bars; large gaps within observed range represented by dashed bars; 95 per cent confidence limit on first appearance represented by medium thickness dark grey bars; 99 per cent confidence limit on first appearance represented by thin light grey bars. These data are also presented in Table 10.1.

monophyletic, since the inclusion of paraphyletic taxa (e.g. ancestors) will lead to an incorrect inference of a ghost lineage (Wagner 1998; Paul, Chapter 5).

Although many of the nodes in the tree presented in Figure 10.2 are relatively weakly supported, the overall structure of the tree is well supported. The second assumption is also justified in that taxa used in the analysis have been scrutinized through character analysis and all exhibit identifiable synapomorphies (e.g. Janvier 1996b). The results of this analysis indicate that although the fossil record of most groups of stem-gnathostomes does not begin until the Silurian, all have ghost ranges that extend a considerable way downwards into the Ordovician (Figure 10.4). This is

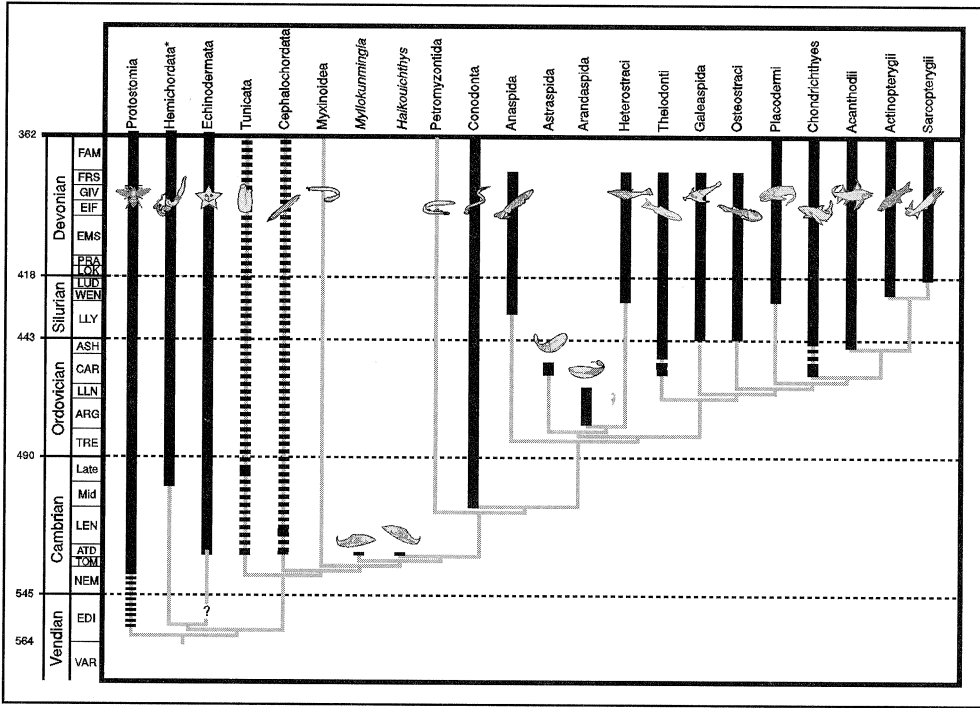


Figure 10.4 Cladogram from Figure 10.2 calibrated to time to reveal inferred ghost lineages; observed stratigraphic range data represented by thick black bars and large gaps represented by thick dashed bars.

surprising given that these organisms have an extensive mineralized component to their skeleton and, thus, might be expected to have a much better fossil record.

### Cladogram fit to stratigraphy

Calibrating cladograms to time and inferring ghost lineages provides a useful visual assessment of the completeness of the fossil record. However, the rigour of this technique has been extended through the application of a number of metrics that assess different aspects of the relationship between tree structure and stratigraphic data. These include the Stratigraphic Consistency Index (SCI; Huelsenbeck 1994), which compares the number of stratigraphically consistent cladogram nodes to the total number of cladogram nodes. The Relative Completeness Index (RCI; Benton and Storrs 1994) attempts to measure the overall level of inconsistency in a tree by quantifying the ghost range implied as the difference between the age of origin of branches subtending sister taxa, divided by the observed range length, and expressed as a percentage. A third metric, the Gap Excess Ratio (GER; Wills 1999) combines aspects of both SCI and RCI, expressing the sum of inferred ghost lineages across a tree as a fraction of the total range of possible ghost lineage values based on a common stratigraphic dataset. Other metrics have been devised but they have not been widely applied, either

without reason (as in the case of the Manhattan Stratigraphic Measure (Siddall 1998), or because of concerns regarding their efficacy, such as Spearman Rank Correlation (SRC; Gauthier *et al.* 1988; Norell and Novacek 1992).

The SRC has been criticized regarding its appropriateness (Huelsenbeck 1994), its contingency upon temporal spacing (Benton and Storrs 1994; Hitchin and Benton 1996), and the procedural requirement of altering the cladogram before analysis (trees must be pectinate for analysis and so more balanced trees must be pruned *a priori* precluding the full analysis; Huelsenbeck 1994). Neither are the SCI and RCI metrics free of potential artefact. The SCI is handicapped by tree balance such that only fully imbalanced trees can achieve the full theoretical range of values 0.00–1.00; perfectly balanced trees also have theoretical maximum SCI score of 1.00, but the minimum value achievable is 0.50 (Siddall 1996, 1997; Wills 1999). The SCI is also affected by the temporal distribution of first occurrences such that if they are all contemporaneous the SCI will equal 1.00, regardless of tree balance; if no first appearances are contemporaneous, the range of SCI is again contingent upon tree balance such that perfectly balanced trees will have a SCI of 0.50, while fully pectinate trees yield the full range of SCI scores (Wills 1999). The effect of tree balance on the RCI is more complex. A perfect RCI score of 100 per cent is possible only if the first appearance of all taxa is contemporaneous and fully pectinate trees fulfilling these stratigraphic requirements will always achieve this score. However, other topologies may not be able to achieve a perfect RCI score even if the component taxa meet these stratigraphic requirements (Wills 1999). The GER controls for the distribution of range data and is also sensitive to tree balance. However, by randomly reassigning the stratigraphic range data over the tree it is possible to assess whether stratigraphy–cladogram congruence is significantly better than random, while holding the potential biases (stratigraphy, taxon number, tree balance) constant (Wills 1999). Permutation tests can also be applied to SCI calculation and the degree to which these metrics deviate from random provides a measure of confidence in their significance (Wills 1999).

The SCI, RCI, and GER (as well as permutation tests for significance of these indices) were calculated for the overall tree and for the internal record of each of the plesions in the overall tree, using Ghosts 2.3 (Wills 1999) and the results are presented in Table 10.2. Dates for chronostratigraphic boundaries used in the stratigraphy file for the program were obtained from Tucker and McKerrow (1995), Gradstein and Ogg (1996), Saylor *et al.* (1998), Tucker *et al.* (1998), Cooper (1999), Encarnación *et al.* (1999), Knoll (2000), and Remane (2000). Internal relationships of the plesions used in the analysis are presented in Appendix 10.2; the full data matrix, as well as the associated stratigraphy files and the occurrence data on which the cladogram–stratigraphy correlation metrics are based, are available from the senior author upon request.

### **Results: internal assessment of the quality of the early chordate fossil record**

The combined results of the analyses outlined above are presented in Figure 10.5 and imply that, overall, the fossil record of early chordates is much better than has been suggested previously. The SCI analysis indicates that approximately two-thirds of the cladogram nodes are consistent with stratigraphic data, and the RCI and GER

Table 10.2 Cladogram–stratigraphy metrics calculated using Ghosts (Wills 1999) for the plesions included in the main analysis based upon hypotheses of relationships included in the appendix

Taxon	n	SCI	SCIsig	RCI	Gmin	Gmax	MIG	GER	GERsig
Echinodermata	16	0.5	0.95	81.319555	349	1552	705	0.70407	0.975
Chordata	18	0.6875	0.99	94.803759	141	1569	282	0.901261	0.99
Conodonts	37	0.685714	0.99	65.305011	177	2258	637	0.778952	0.99
Anaspida	5	0	0	-33.333333	15	60	60	0	0
Heterostraci	18	0.625	0.99	47.826087	67	965	132	0.927617	0.99
Thelodonti	4	0	0	48.076923	24	54	54	0	0
Galeaspida	7	0.461538	0.876	35.97561	33	346	105	0.769968	0.99
Osteostraci	25	0.5	0.435	63.186813	46	214	134	0.47619	0.94
Placodermi	22	0.55	0.713	61.977186	36	400	200	0.70896	0.993
Chondrichthyes	14	0.5	0.606	72.643375	140	552	415	0.332524	0.736
Acanthodii	8	0.5	0.525	66.924565	61	282	171	0.502262	0.75
Actinopterygii	15	0.230769	0.681	-97.321429	356	777	442	0.795724	0.99
Basal Synapsida <sup>a</sup>	13	0.727273	0.975	80.96	61	462	107	0.885287	0.5
Basal Diapsida <sup>b</sup>	8	0.83	1	40.3	61	241	74	0.927778	0.5

'n' is the number of terminal taxa that the metrics are based upon, 'SCI' is the Stratigraphic Consistency Index (Huelsenbeck 1994), 'SCIsig' is the significance that the SCI value is better than random, 'RCI' is the Relative Completeness Index (Benton and Storrs 1994), 'Gmin' and 'Gmax' are the minimum and maximum possible summation of the temporal ranges of the terminal taxa included in the analysis based upon a rearrangement of the terminals such that they achieve best- and worst-possible fit to stratigraphy. 'MIG' is the Minimum Implied Gap based upon the given topology of relationships and stratigraphic data, 'GER' is the Gap Excess Ratio (Wills 1999) and 'GERsig' is the significance that the RCI and GER values are better than random. The basal synapsid and diapsid metrics were calculated as part of a study by Benton and colleagues, including Benton and Hitchin (1996), and further details can be found at the following url: <<http://palaeo.gly.bris.ac.uk/cladestrat/reptiles.html>>.

<sup>a</sup> Based on the hypothesis of relationships from Modesto (1995, fig. 19A).

<sup>b</sup> Based on the hypothesis of relationships from de Braga and Reisz (1995, fig. 6).

analyses both indicate that the record is approximately complete. Permutation tests reveal that these values are not significantly worse than random ( $P > 0.99$ ). However, analyses of the stratigraphic data and their correlation to cladograms of the internal relationships of the operational taxa indicate that the quality of the record varies from group to group. For instance, the fossil record of the living jawless vertebrates and invertebrate chordates is so poor that at 95 per cent confidence the first appearance of these groups can only be constrained within an interval that predates the origin of the Earth in some groups (lampreys), and the origin of the universe in others (hagfishes). This is not an altogether surprising result given that these organisms are entirely soft-bodied and the chances of their preservation in the fossil record are very low. However, the same cannot be said for taxa more derived than lampreys, all of which possess a mineralized, and therefore readily fossilizable, component to their anatomy. Again, the fossil record of these groups is of variable quality and the metrics offer conflicting interpretations of the dataset. For instance, confidence limits suggest that our knowledge of the conodont fossil record is very mature; at 95 per cent confidence the first appearance of the group suggests that it lies within a bracket of 346 kyr of the first stratigraphic appearance, and at 99 per cent confidence, within a bracket of 532 kyr of this datum, both of which are beyond the limits of stratigraphic resolution within this interval.

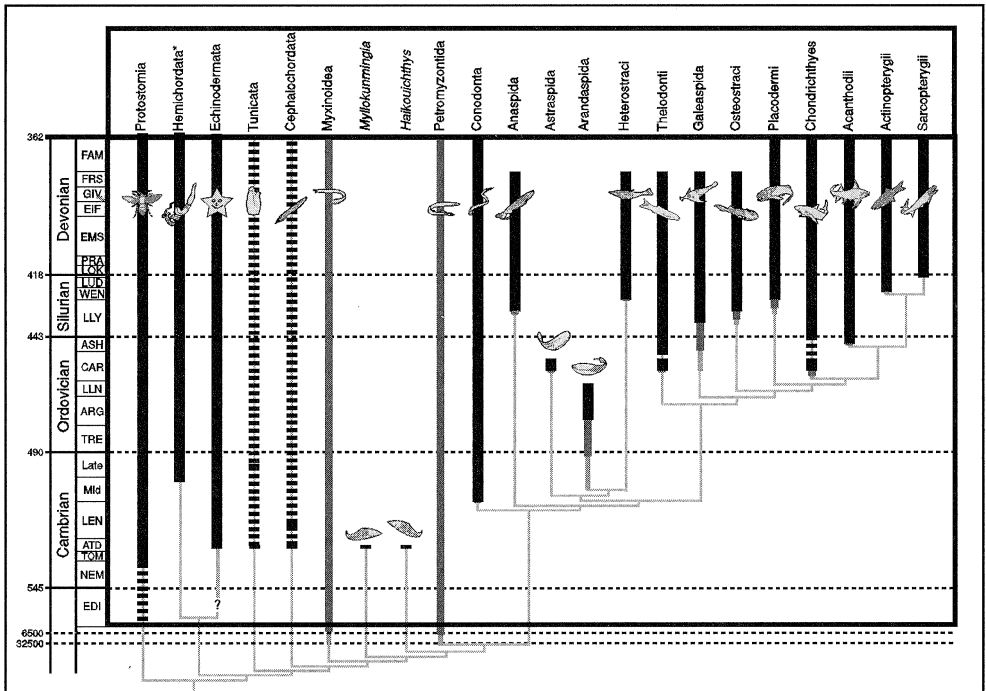


Figure 10.5 Cladogram from Figure 10.3 calibrated to time and including 95 and 99 per cent confidence intervals on stratigraphic range data; medium thickness dark grey lines represent 95 per cent confidence intervals and thin light grey lines represent 99 per cent confidence intervals.

The GER value is less supportive, but nevertheless indicates that the cladogram–stratigraphy correlation is close, within 0.77 of a tree constructed solely on the basis of stratigraphic order of appearance. However, the other cladogram–stratigraphy metrics suggest that the record is only moderately complete; the SCI indicates that only two-thirds of the cladogram nodes are stratigraphically consistent and the RCI suggests that the known record is only two-thirds complete. All values achieve 99 per cent confidence that they are no worse than random. Taking another example of the better groups, assessments of the heterostracan fossil record have also yielded conflicting results; at 95 per cent confidence, the bracket on first appearance is only 278 kyr, and 432 kyr at 99 per cent confidence. This compares well with the GER which indicates that cladogram–stratigraphy correlation is within 0.92 of a tree perfectly concordant to the stratigraphic data. However, the SCI and RCI metrics tell a very different story: less than two-thirds of cladogram nodes are stratigraphically consistent and the heterostracan fossil record represents less than half of the hypothesized evolutionary history of the group.

Although both examples show the same conflicting pattern between metrics, they probably result from two different artefacts in the datasets. Knowledge of the intrarelationships of conodonts is at a relatively immature stage and all existing schemes

are based on stratophenetic analysis of the dataset. It is surprising, therefore, that there is not a better tree–stratigraphy correlation. However, in the process of converting the published phylogeny (Sweet 1988, and in Sweet and Donoghue 2001), some ‘hard’ polytomies have been converted to ‘soft’ polytomies for the purposes of analysis and, thus, ghost ranges have been artificially extended and statistical scores are artificially lower than might be expected. On the other hand, the intrarelationships of heterostracans have been analysed independently of stratigraphic data (Blieck 1984; Blieck *et al.* 1991; Janvier and Blieck 1993; Janvier 1996b) and although the cladogram–stratigraphy correlation of well-understood groups is good, the SCI and RCI metrics are depressed because many taxa are too poorly known to be included in phylogenetic analyses and have, thus, been placed in a soft polytomy in the most derived position likely, based on character distribution across existing trees. However, given the method of tree construction, it is likely that the short confidence interval and high GER value provide a better assessment of the completeness of the heterostracan fossil record than do the SCI and RCI metrics. In short, the fossil record of both groups is probably very complete but relatively poorly understood, the lack of understanding arising from poor quality data in heterostracans and from poorly resolved relationships in both groups (cf. Benton *et al.* 1999).

Conodonts and heterostracans are important for providing constraints on molecular estimates for the divergence of the living jawless vertebrates and jawed vertebrates, and it is therefore a happy coincidence that they appear to possess a fossil record that exhibits internal consistency. Assessments of the quality of the record of groups that might constrain the divergence of crown-group jawed vertebrates suggest that implied divergence dates may be less reliable. Groups such as the osteostracans, placoderms, and chondrichthyans bracket this diversification event, and potentially provide important upper bounds on divergence timing. However, cladogram–stratigraphy correlation in these groups is poor, generally at a level of 50 per cent for the SCI, at  $\leq 0.5$  for the RCI (except placoderms which appear to have a fossil record that is internally more consistent), and with a GER  $\approx 0.3$ – $0.5$ ; SCI values do not pass a 95 per cent confidence test to determine whether they are not significantly worse than random, although GER and RCI values are generally no worse than random at the same confidence level. Positive correlation between high and low GER and SCI values suggests that poor cladogram–stratigraphy correlation does not arise solely from cladogram inaccuracy, which would normally produce an inverse correlation, although cladogram inaccuracy is possibly an important factor. It is more likely that correlated low GER and SCI values reflect a genuinely poor fossil record and this is corroborated by relatively long confidence intervals on stratigraphic occurrence data in, for example, chondrichthyans, which have a confidence bracket of  $>1.8$  myr. However, this calculation is based on the compilation of Ordovician and Silurian occurrences, only 9 of the 67 of which are Ordovician, and these occurrences are limited to a narrow interval in the Caradoc (Harding Sandstone and its equivalents). Thus, the fossil record of this group appears to be particularly intermittent early on, and a reassessment of confidence limits on first appearance based upon the Ordovician record alone results in a bracket of over six million years. The same situation is true of thelodonts. Inverse correlation between low SCI ( $P > 0.95$ ) and high GER ( $P < 0.95$ ), as in the placoderms and actinopterygians, probably results from a good, but poorly understood, fossil record. The fossil record of placoderms is rich, but attempts to resolve the

relationships of the group have thus far proved only variably successful (e.g. Goujet and Young 1995; Goujet 2001). The fossil record of actinopterygians is more gap than record, hence the strongly negative RCI ( $P < 0.99$ ), but the sum of implied ghost ranges is very low compared with the maximum, and very close to the minimum possible by optimizing stratigraphic fit/discordance to the tree (GER 0.79; same  $P$  as for RCI).

### **Problems with assessing the quality of the record**

There are two potential problems with regard to this analysis, one relating to the analysis itself, and the second relating to potential artefact in the dataset. First, there is a very poor correlation, absolutely and proportionally, between the confidence intervals on each of the groups, which are derived from internal assessments of the quality of the record within each of the plesions, and the inferred ghost lineages, which are based on analysis at plesion level (compare Figures 10.4 and 10.5). Paul (1998) suggested that this may be an appropriate means of identifying ghost lineages that are an artefact of cladistic methodology, rather than reflecting a true gap in the temporal record of a lineage. We outlined earlier why we think that our analysis is not subject to this kind of artefact (plesions are monophyletic).

The second problem relates to the dataset and has implications for the analysis of confidence intervals and, in turn, their degree of fit to ghost lineages. The calculation of classic confidence intervals assumes that fossil recovery potential is random. Testing this assumption is very difficult when dealing with global compilations of palaeontological data and probably represents the greatest limitation upon the extension of confidence intervals to global datasets. Nevertheless, there is some evidence to suggest that there are two significant biases in the dataset, indicating that the existing dataset is not a random sample of the fossil record. First, the vast majority of known occurrences are from northern Europe, the USA, and South-East Asia, compared with a global fossil collection bias for north-west Europe and North America (e.g. Smith 2001). Although there are numerous fossil records from North America as a whole, the vast majority of taxonomic treatments of North American faunas (especially Arctic Canada) are new taxa, suggesting that although the North American record is being recovered rapidly, it has been sampled only sparsely to date (using the collecting curve analogy we remain on the steep component of the curve). A bias against collecting central Asian faunas appears to be supported by records of spot occurrences in terranes such as Tuva (Afánassieva and Janvier 1985). A virtual absence of 'ostracoderm' faunas, bar thelodonts, from Gondwana after the Ordovician may also suggest a dearth of collecting. However, many basins have been densely sampled, particularly for conodont biostratigraphy, to little avail (the exception to this being the enigmatic pituriaspids; Young 1991). It would appear that the absence of records from this interval does reflect the real absence of most 'ostracoderm' groups in Gondwana during this time (for further discussion see Smith *et al.* 2002). Thus, there is a systematic bias in the sampling of geographical regions, but some gaping holes in the regional distribution of fossil sites result from primary signal rather than an absence of sampling.

Another source of evidence supporting a non-random fossil record stems from the fact that the distribution of many, or even most, groups was facies controlled. Given the differential preservation potential of facies with sea level change, it would be expected

that the recovery potential and, thus, the stratigraphic distribution of facies-controlled fossil taxa would be similarly affected (Holland 1995). While the only recourse to removing a geographical collecting bias is systematic sampling of unsampled regions, the effect of a non-random record upon the calculation of confidence intervals on stratigraphic data may be readily overcome, at least in principle. This is achieved through abandoning the uniform recovery potential assumption of classic confidence limits (Paul 1982; Strauss and Sadler 1989; Marshall 1990) and replacing it with a fossil recovery potential function that reflects secular bias resulting from, for example, sea level change (Holland 1995; Marshall 1997). Devising this function can be non-trivial, but in many cases it may be simplified on the basis that it is only change with stratigraphic position that is significant (Marshall 1997).

Our attempts to implement the 'generalized' method of calculating confidence intervals failed on a number of counts. First, the method requires that the stratigraphic position of each fossil occurrence is known with a degree of precision that is not possible with the global dataset of early vertebrates; the stratigraphic position of some occurrences cannot be resolved even to series level. Second, fossil recovery potential functions are incalculable at the taxonomic level at which our analysis has been undertaken. Many of the component lineages (e.g. heterostracans and osteostracans) exhibit an ecological shift through time and phylogeny (Blieck and Janvier 1991; Smith *et al.* 2002) and so it would have been necessary to divide plesions into much lower taxonomic levels for which fossil recovery potential curves could be produced and implemented. The conflation of these two variables precluded analysis of the entire dataset. As a fallback, and given that it is the time of first appearance of groups that is germane to this study, it was our intention to confine application of the generalized method to the pre-Silurian record alone. This objective is more easily achieved because the secular distribution of vertebrates is much better constrained for the Cambro-Ordovician (mainly because the records are entirely marine), and the ecologies of taxa are less complex than for post-Ordovician vertebrates. However, while it is possible to derive fossil recovery probabilities for each lineage, the calculation of fossil recovery potential functions is precluded by almost total absence of agreement over a eustatic sea level curve for the interval. While we intend to remedy this problem in the near future, it is beyond the scope of the present study. In the interim, we have observed elsewhere (Sansom *et al.* 2001; Smith *et al.* 2002) that intracontinental occurrences of Ordovician vertebrates in Laurentia are confined to eustatic highstand episodes. Thus, although it has not proved possible to quantify confidence intervals that consider systematic bias in groups that have their first records in the Ordovician, we may conclude that the base range of the Ordovician groups (bar conodonts) would be revised downwards. To provide constraint on the lower limit of first appearance we note that the absence of records from preceding highstand episodes is significant.

## **Comparison of molecular and fossil estimates**

### ***Origin of chordates, craniates, and vertebrates***

Inferences regarding the time of origin of these clades are hampered by the perennial problem of first appearances clustering in the Atdabanian (mid-Early Cambrian). The absence of outgroup representatives of greater age precludes further interpretation



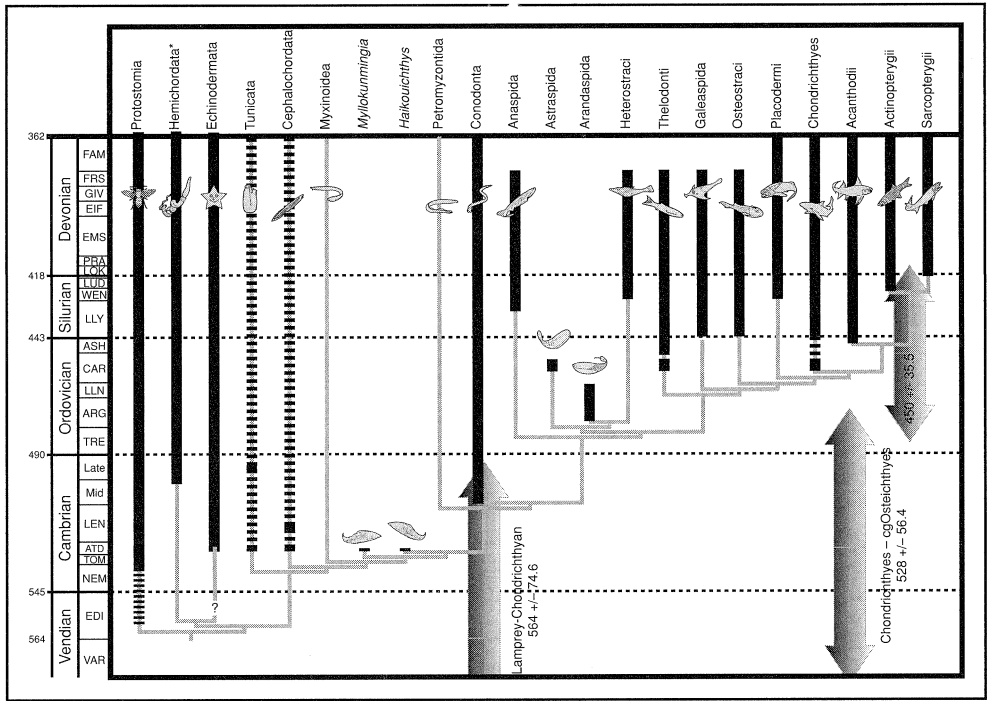


Figure 10.6 Observed stratigraphic range data including inferred ghost lineages and molecular estimates on the divergence of various clades (from Kumar and Hedges 1998).

beyond the conclusion that representatives of these clades are observed, or can be inferred to have been present, at this time (530 Ma). There are two reasons for equivocation over this date. First, putative echinoderm remains are known from the Proterozoic (e.g. *Arkarua* Gehling 1987), possibly providing evidence for a chordate ghost lineage extending back to the Neoproterozoic. Second, the fossil record of these groups is so poor that internal assessments of confidence limits ( $P > 0.95$ ) place a bracket on the evolutionary origin of the cephalochordates, hagfishes and lampreys that is sufficiently broad to encompass any hypothesis that is compatible with their origin within the constraints provided by the origin of the Earth and/or Universe, as well as some that are not. Thus, without recourse to negative evidence, the fossil record is mute with regard to a judicious lower constraint on the timing of origin of chordates, craniates, and vertebrates.

The absence of firm palaeontological data is unfortunate because molecular estimates for the diversification events are strongly discordant with the available evidence (Figure 10.6), although molecular estimates also differ from one another by just as great a degree. The earliest molecular estimate for the divergence of chordates from their sister clade, the Ambulacraria (echinoderms plus hemichordates), is 1001 Ma (Wray *et al.* 1996), while the latest is 590 Ma (Feng *et al.* 1997). The average estimate is 722.75 Ma with a standard deviation of 192 myr ( $n = 4$ ). The time of divergence of craniates and acraniates has been addressed in only two analyses, yielding

estimates of 700 Ma (Nikoh *et al.* 1997) and 751 Ma (Hedges 2001). These analyses are non-independent in that the sequences used by Nikoh *et al.* (1997) were also used by Hedges (2001), but yielded results from individual sequences that differ by as much as 100 Ma that can be accounted for by differences in calibration dates and analytical techniques (Hedges 2001). The calibration points used by both analyses are questionable in that they use molecular estimates and, thus, do not provide an adequate or independent test of molecular clock theory. Furthermore, the calibration date for the divergence of arthropods and vertebrates (700 Ma) used by Nikoh *et al.* (1997) is derived from Dayhoff (1978) who provided no form of substantiation for a date that does not accord with any palaeontological data. In addition, the use of molecular calibration points in this analysis appears to render the analytical argumentation circular since the molecular calibration points are applied to molecular sequences that were used in calculating the molecular estimate. Specifically, Hedges (2001) uses calibration points derived from Kumar and Hedges (1998; and Wang *et al.* 1998) that are based, in part, upon aldolase and TPI, which are analysed in Hedges (2001). Given that the only palaeontological calibration point used by Kumar and Hedges (1998) is the bird–mammal divergence date of 310 Ma, Hedges (2001) has, in effect, calibrated multiple times, directly and indirectly, using a single palaeontological datum.

No molecular estimates have been calculated for the divergence of invertebrate craniates and vertebrates. This is because molecular phylogenies consistently resolve hagfishes and lampreys as monophyletic and, hence, there is no distinction between craniates and vertebrates within the field of molecular systematics. However, Hedges (2001) used a subset of his data to provide an estimate for the divergence of hagfishes and lampreys within the context of cyclostome monophyly and arrived at a date of  $499 \pm 36.8$  Ma, using the lamprey–gnathostome molecular estimate of 564 Ma (Kumar and Hedges 1998) as the calibration point. For once, palaeontological data indicate two older spot dates (530 Ma on *Myllokunmigia* and *Haikouichthys*) with a slightly younger and better-constrained date of 495 Ma bracketing the molecular estimates and falling well within the standard error on the molecular estimate.

Overall, in consideration of the origin of chordates and divergence of the lower chordate groups, there is poor correlation both between molecular and palaeontological estimates, and between molecular estimates that are based upon different datasets. Thus, in the absence of any independent constraint that the fossil record may otherwise afford, it would be dangerous to conclude anything other than the fact that chordates, craniates, and vertebrates had diverged by 530 Ma. Furthermore, given the low stochastic value of the calculated error limits on published molecular clock studies, there can be no confidence that the origin of the clade was not significantly before such estimates, based on molecular data alone.

### **Origin of gnathostomes**

Although there is little temporal constraint on the origin of vertebrates afforded by the fossil record, internal assessments of the consistency of the record point to a dramatic increase in the quality of the record after the divergence of the lineages leading to lampreys and living jawed vertebrates (crown-gnathostomes). This coincides with the appearance of mineralized skeletonization within the gnathostome

stem-lineage. The fossil record of conodonts exhibits remarkable internal consistency and the first appearance of this basal member of the gnathostome stem-lineage affords an inferred 495 Ma constraint on the latest possible date for divergence, with a confidence bracket of just 346 kyr ( $P > 0.95$ ; and only 532 kyr with  $P > 0.99$ ). The absence of a sister taxon with even an approximately comparable fossil record precludes the possibility that this date can be corroborated through inference of a ghost lineage. The absence of a stepwise geological appearance of successive sister taxa within the sister clade to the Conodonta is problematic; the conodont fossil record implies a significant ghost lineage amongst many of these groups. This signal is in agreement with internal assessments of the quality of the record of these groups (which conclude that it is a poor reflection of the evolutionary history of these groups), the fact that all well-known taxa fall within known clades within the stem-lineage (rather than as individual plesions on the stem-lineage), and knowledge of a large number of vertebrate remains of this age that cannot be assigned to known groups (e.g. Sansom *et al.* 2001).

Only two molecular clock analyses have addressed the lamprey–gnathostome divergence. Wray *et al.* (1996), who famously estimated the divergence of Bilateria at 1200 Ma, estimated the divergence of lampreys and gnathostomes at 599 Ma, while Kumar and Hedges (1998) suggested a date of 564 Ma. The entire analysis undertaken by Wray *et al.* (1996) was reanalysed and robustly criticized by Ayala *et al.* (1998), who revised upwards all the divergence estimates. This is in accordance with the analysis undertaken by Kumar and Hedges (1998) which, given the enormity of the dataset, must be considered the most robust analysis undertaken to date. Despite the poor internal support for palaeontological dating of the origin of gnathostomes, the average date given by Kumar and Hedges (1998; 559 Ma) corresponds well to the palaeontological estimate, at least when considered within the context of a standard error of  $\pm 74.6$  myr on the molecular estimate which encompasses an interval extending from the earliest Ordovician to the Neoproterozoic (484.4–633.6 Ma).

### **Origin of crown-group gnathostomes**

Inference of the time of origin of crown-gnathostomes is complicated by equivocation over the affinity of shark-like microremains from the Late Ordovician and early Silurian (for a discussion see Janvier 1998; Sansom *et al.* 2000, 2001; Smith *et al.* 2002). These scales are identified as chondrichthyans (total-group Chondrichthyes) on the basis that they possess neck canals and exhibit rigidly patterned areal growth, at present a chondrichthyan synapomorphy and symplesiomorphy, respectively. However, it is not known whether the inclusion of these taxa renders the group paraphyletic or, indeed, whether these characters are exclusive to crown-gnathostomes; only further resolution of the anatomy of these taxa will lead to a resolution of their precise placement within total-group gnathostome systematics. In the meantime, all available evidence suggests that they are representatives of total-group Chondrichthyes and, thus, crown-gnathostomes; by inference they place a lower constraint on the divergence of crown-gnathostomes at 457 Ma, but with a confidence interval extending to 463.4 Ma ( $P > 0.95$ ; accepting the confidence interval based upon the Ordovician record alone).

A molecular estimate for the time of divergence between Chondrichthyes and Osteichthyes, and hence, the time of origin of crown-gnathostomes, was calculated by Kumar and Hedges (1998) at  $528 \pm 56.4$  Ma, encompassing an interval from the mid-Ordovician (471.6 Ma) to late Neoproterozoic (584.4 Ma). The palaeontological estimate derived from a literal reading of the record (457 Ma) narrowly misses the lower bound on the bracket provided by the standard error on the molecular estimate. Confidence limits provided by the Ordovician record alone extend the predicted first appearance closer to the lower bound on the molecular estimate, but the two do not overlap.

### **Origin of actinopterygians and sarcopterygians**

The earliest actinopterygian remains are dated at 425 Ma, and are part of a rich record (thanks, again, to the application of micropalaeontological techniques; e.g. Schultze 1968; Gross 1969; Mårss 1986; Fredhölml 1988a,b). This confers a remarkably short confidence interval (425.58 Ma at  $P > 0.95$ ) on first appearance. The record of pre-Devonian sarcopterygians is much poorer, with only a single known fossil horizon (Zhu and Schultze 1997). With such a poor record, the obvious implication is that the true range extends much further back than present evidence indicates. This is supported by the observation that these occurrences were palaeogeographically remote from each other. However, this earliest sarcopterygian record (423 Ma) is remarkably consistent with the extent of the range of actinopterygians such that there is very little inferred ghost lineage. Thus, we accept 425.58 Ma as a firm lower bound on the origin of crown-osteichthyans and the divergence of two osteichthyan clades.

Kumar and Hedges' (1998) estimate for the divergence of the two extant osteichthyan clades is  $450 \text{ Ma} \pm 35.5 \text{ myr}$ , encompassing an interval extending from earliest Ordovician (485.5 Ma) to Early Devonian (419.5 Ma). This compares well with the palaeontological estimate, although internal assessments of the quality of the record, particularly of sarcopterygians, suggest that palaeontological data may eventually converge on the mid-range of this molecular estimate.

### **Discussion**

Correspondence between molecular clock estimates for the timing of divergence and palaeontological data indicating the minimum possible date for divergence is very variable. There is a clear correspondence between molecular and palaeontological estimates where there is *a priori* evidence for confidence in the fossil record based upon internal assessments of its quality based upon stratigraphic data alone and the relationship between stratigraphic data and cladogram structure (e.g. the lamprey-gnathostome and actinopterygian-sarcopterygian divergences). Concomitantly, where there is *a priori* evidence for a lack of confidence in the quality of the record on the basis of internal assessments, the molecular and palaeontological estimates are in discord.

Where there is disagreement between palaeontological and molecular estimates it is difficult to reconcile which dataset provides the best approximation of true time of divergence of a particular clade. Palaeontological estimates are limited by their reliance upon negative evidence and although quantitative methods are being developed to

assess the plausibility of range extensions in the face of sampled, but barren time intervals (Weiss and Marshall 1999), they are at present limited by the assumptions on which they are based, many of which are extremely controversial. On the other hand, given that it is difficult to reconcile between competing molecular estimates, it is not surprising that it is difficult to arbitrate between palaeontological and molecular estimates. This is partly because, as scientific theories, molecular clock calculations are extremely poorly formulated and, thus, are difficult to test. In many instances, one molecular hypothesis is preferred over another on the basis that it is derived from the greatest dataset, relying upon a law of large numbers approach to molecular clock mechanics (cf. Rodríguez-Trelles *et al.*, Chapter 1), rather than a neutrality theory basis (Zuckermandl and Pauling 1962, 1965). Thus, they are testable only by other molecular clock calculations, based upon larger, more universal datasets and/or the falsification or augmentation of calibration points. Smith and Peterson (2002) have suggested an explanation for the discrepancy between molecular and palaeontological temporal divergence estimates, arguing that they reflect two quite distinct events, with molecular clocks estimating the time of origin of a clade, and palaeontological estimates recording the diversification of the clade, which they equate to the origin of the total-group and origin of the crown-group – placing undue weight on the evolutionary significance of crown-groups. This follows the widespread assumption that most molecular clock estimates pertain to total-group divergence, but total-groups and crown-groups are hierarchical such that one taxon's total-group is the next more inclusive taxon's crown-group and *vice versa*. Thus, there is no better correlation between molecular and palaeontological estimates for the origin of crown-groups than total-groups, and the rapprochement fails.

Even when palaeontological and molecular estimates are comparable, molecular clock analyses consistently yield a date that is considerably older than the palaeontological data indicates (except in the instance of the hagfish–lamprey divergence estimate within the context of cyclostome monophyly). Thus, the fossil record of early chordate evolution is either consistently missing the early history of various chordate clades or molecular clock dates consistently overestimate the true time of cladogenesis. To some extent this should be expected. First, because no one argues that the earliest fossil record equates to the origin of a clade; there is a cryptic evolutionary history to all clades, the critical issue is the temporal extent of this period of unrecorded evolutionary history. Second, in a strict interpretation of molecular clock theory, such calculations estimate the time of divergence based on a fossil record comparable with that on which the 'clock' is calibrated, not the true time of origin of a clade and, hence, it has been argued that molecular clock estimates should be conservative. All of the clades in our analysis exhibit stratigraphy–cladogram congruence metrics that are worse than the fossil record of the calibration point on which most molecular clocks are calibrated, the divergence of bird–mammal lineages at 310 Ma (Table 10.2; but see Lee 1999; it should also be noted that although the basal synapsid and diapsid fossil records perform well in the SCI and GER indices, it is likely, given the patchy nature of the record – betrayed by the particularly low RCI for the diapsid lineage – that confidence intervals on the stratigraphic range data for the various plesions would be extensive).

However, there is some circumstantial evidence to suggest that the fossil record of early vertebrates, and total-group gnathostomes in particular, may be more reliable

than we would otherwise assume. This stems from the rather surprising degree of correspondence between molecular estimates and palaeontological data, at least in terms of the chronologically consistent ordering of palaeontologically based estimates for the first appearance of successive extant clades. While molecular estimates for the divergence of successive clades have to be chronologically consistent, by definition (they are based upon a direct extrapolation from a hierarchical dataset), the same does not hold true for the fossil data. Indeed, the temporal distribution of fossil remains will only be chronologically consistent if their ordering reflects the hierarchy of evolutionary relationships – which they will do only if the fossil record is preserved with high fidelity. There is also evidence to suggest that molecular clocks may consistently overestimate the date of divergence of clades. This can occur for two non-mutually exclusive reasons. First, constraints on molecular clock estimates are asymmetrical, i.e. they are bound to be non-negative but there are no such constraints at the upper end of the spectrum (Rodríguez-Trelles *et al.* 2002). Second, overestimation of divergence timing arises from the accumulating inaccuracy associated with extrapolating farther and farther from the calibration date (Springer 1997; Nei *et al.* 2001) and especially concerns analyses that use single internal palaeontological calibration dates, although it also affects analyses that use multiple external and/or internal calibration dates that are derived from a single palaeontological calibration date. This may be a particular weakness of the analysis by Kumar and Hedges (1998); although encompassing by far the greatest number of sequences in calculating divergence times (658), very few of these were used in calculating the timing of the very oldest divergence events (13 for the origin of jawed vertebrates, 15 for the origin of crown-gnathostomes, 44 for the origin of crown-osteichthyans).

The alternative view, that the fossil record of early chordates is particularly poor, is reflected by the fact that plesiomorphic chordate and deuterostome anatomies have been the subject of debate for over a century and yet the subject remains resolutely intractable. In addition, very few stem-chordates, stem-ambulacrarians, and/or stem-deuterostomes have been identified from the fossil record (e.g. Jefferies *et al.* 1996; Gee 2001; Shu *et al.* 2001b) and none (arguably) have escaped critical examination (Ruta 1999; Lacalli 2002). Finally, questions of chordate, craniate, and vertebrate divergence timings cannot be resolved in isolation while debate over the veracity of the Cambrian ‘explosion’ continues (e.g. Smith 1999; Budd & Jensen, Chapter 9).

The other molecular estimate that exhibits poor correspondence to palaeontological data, the divergence of crown-gnathostomes, predicts a Late Cambrian event and, by inference, a hitherto unrecorded interval of crown-gnathostome evolutionary history that spans the Late Cambrian–Middle Ordovician. Significantly, this interval coincides with recent discoveries of a swathe of new vertebrate taxa, which are assignable to the gnathostome total-group, but no further, based upon the available evidence (Sansom *et al.* 2001). It is quite possible that these new taxa include further Ordovician representatives of crown-gnathostomes (e.g. *Skiichthys* Smith and Sansom 1997). The Late Cambrian–Middle Ordovician gap in the record overlaps well with gaps in the records of other groups, for example, the echinoderms (Smith 1988a). This hints at a secular bias in the fossil record as a whole that probably reflects the fact that imperfections in the fossil record are rooted in imperfections in the rock record (cf. Holland 1995, 2000; Smith 2001; A.B. Smith *et al.* 2001).

## **Implications for established hypotheses and scenarios**

### ***Understanding early chordate evolution using an incomplete fossil record***

If nothing else, molecular clocks have provided the stimulus for palaeontologists to look at their datasets anew and provide justification for cherished methodologies. This, in turn, has provided the impetus for the development of old and new methods for assessing the completeness of the fossil record. These internal assessments provide a means of determining degrees of confidence in subsets of a dataset, provide cautionary limits in reading the evolutionary history of particular clades, and provide predictions in our attempts to recover missing components of the record.

The greatest concern of palaeontologists with regard to the mismatch of molecular clocks and the fossil record may be that it indicates not only that the fossil record is substantially incomplete but also, critically, that it is the initial period of the evolutionary history of these clades that is missing. In such a case, would not the usefulness of the fossil record, in uncovering the sequences of character change between extant clades, be compromised? The simple answer to this question is no. The chief value of the fossil record is that it reduces error in inferring the sequence of character changes that underlie the establishment of living clades – this has been integral to testing and rejecting models such as, for example, the origin of paired appendages within vertebrates (Coates 1994). Whether or not we have a complete sample of the anatomical designs that have been realized is not relevant; with the fossil record we have a more complete, and continually expanding, understanding of chordate evolution than would be possible using only the living biota. Furthermore, fossils help to prevent the identification of homoplasy as homology in living members of distantly related groups, and identify homologies that might not otherwise be recognized because of the hundreds of millions of years of evolutionary change that has occurred subsequent to the divergence of the clades.

Our understanding of early chordate evolution may well be incomplete but it does not follow that it is incorrect. Further attempts to reconcile the fossil record with the living biota will lead to further refinement not only to the temporal scale of early chordate evolution but also to our understanding of the sequence of character changes that shaped all subsequent events in chordate phylogeny.

### ***Neoproterozoic refugia and the origin of vertebrates***

One inevitable development of molecular clock estimates is that attempts are being made to link intrinsic evolutionary change to extrinsic environmental factors. For instance, van Tuinen *et al.* (1998) proposed that the origin of ratites is not just coincident with, but inextricably linked to, the separation of Africa and South America during the Early to mid-Cretaceous (see Cracraft 2001 for an excellent analysis). More recently, Hedges (2001; Chapter 2) proposed that the proximity of the molecular clock estimate for the divergence of crown-vertebrates to radiometric dates for the first major Neoproterozoic glaciation (Sturtian; 750–700 Ma) may not be coincidental. It is argued that both the Sturtian and Varanger glaciations (610–570 Ma) would have led to contraction in the topological range of species and, through long-term genetic isolation in small refugia, to considerable speciation. As worthy as this approach may be in demonstrating an integrated approach to the questions of when, where, how,

and why vertebrates first evolved, there are two significant problems with regard to this linkage of intrinsic evolutionary and extrinsic environmental factors. First, the nature, timing, and tempo of the Cryogenian period of the Neoproterozoic is utterly unresolved, in terms of the timing, duration, and number of glaciation episodes (Knoll 2000). Second, and more intractably, the standard errors on molecular estimates are currently so vast (and unrealistically conservative) that they render worthless any attempt to match biotic events to radiometrically dated environmental events.

### ***Evolutionary scenarios based upon palaeontological dating***

Although molecular estimates fail to provide the necessary temporal constraint to underpin attempts to uncover any possible link between intrinsic evolutionary events and extrinsic environmental events, palaeontological data provide no panacea either, at least with regard to the origin and early evolution of vertebrates. It has been recognized for many years that evolutionary history cannot be read directly from the rocks, but many scenarios for the origin of major clades remain current, even though the supporting data have not expanded from those on which they were originally contrived. For instance, Romer's celebrated 'eurypterid influence on vertebrate history' (Romer 1933) is based upon the co-occurrence and vaguely comparable diversity trends of eurypterids and the then earliest skeletonizing vertebrates in the Silurian. Thus, the origin of the skeleton has been attributed to the selection-based effect of predating eurypterids upon early vertebrates. But not only are the earliest known skeletonizing vertebrates now Cambrian in age, and the earliest undisputed 'armoured' vertebrates Ordovician in age, but our phylogenetic tests and internal assessments of the consistency of stratigraphic data both reveal that these lineages probably existed even earlier. Thus, the co-occurrence and evolutionary history of vertebrates and eurypterids is no longer apparent and Romer's evocative theory must finally be laid to rest.

Similarly, it has been argued that the rise of jawed vertebrates and apparently concomitant demise of skeletonizing jawless vertebrates is the result of competitive displacement (for a summary see Purnell 2001). However, our analyses reveal an extensive cryptic history of early jawed vertebrates that has not been considered in the formulation of the theory, or in attempts to test it. Furthermore, it may not be possible to test such hypotheses adequately on the basis of the currently available dataset.

The bottom line with regard to attempts to link intrinsic and extrinsic events in early vertebrate evolution is that although there are many interesting questions that can be asked, it may not be appropriate to try and answer some of them based upon the available palaeontological dataset, and molecular clock analyses do not at present appear to be even close to capable of overcoming these shortcomings.

### **Conflict, compromise, or consilience?**

Increase in the application of molecular clock theory has led to a considerable period of introspection amongst the palaeontological community, from which two main camps have emerged. There are those who reject molecular clock estimates outright and contend that only the fossil record can provide reliable estimates for the divergence of clades, albeit minimum estimates for the timing of divergence events (e.g. Conway Morris 1997, 2000; Budd and Jensen 2000). Others have capitulated entirely to molecu-



lar clock estimates, concluding that use of the fossil record is corrupted by its reliance upon negative evidence (Fortey *et al.* 1996, 1997; Smith 1999; Wills and Fortey 2000; Smith and Peterson 2002). However, neither dataset has a monopoly over the other and, indeed, the two datasets have much mutuality. The inextricable linkage between the fossil record and molecular clock theory is no better exemplified than in the need for palaeontological calibration points in molecular clock analyses, whether they are applied directly or indirectly. Above all, the two databases provide a level of rigour that would not be possible in the absence of one or other dataset, such that molecular clock theory and the fossil record are becoming better understood through reciprocal illumination.

Given the degree of latitude offered by standard error on molecular clock estimates and the lack of internal consistency in the fossil record of early chordates, we are no closer to constraining the times of origin of the chordate, craniate, and vertebrate clades. Indeed, it could be argued that we are even further from providing constrained estimates on the origin of these clades than we were at the outset. Thus, although we understand relatively well what is currently known of early chordate evolution, it appears that what is currently known is by no means all there is to know, and this is particularly the case for the invertebrate chordates, basal vertebrates, and stem-gnathostomes within the Late Cambrian–Middle Ordovician, and lower Silurian intervals. While our knowledge of the invertebrate chordate component of chordate phylogeny will remain contingent upon the chance discovery of fossil remains preserved under exceptional conditions, such a restriction does not obtain for the skeletonizing vertebrates, the remains of which were readily entrained in the fossil record. Targeted examination of previously unsampled environments and palaeogeographical realms will be crucial to resolving the evolutionary history of early vertebrates and stem-gnathostomes in particular. At the same time, development of molecular clock theory, more rigorous composition of molecular clock analyses as scientific hypotheses for testing, and the inclusion of more sequences representative of basal chordates and sister groups are likely to provide better constraints on their time of origin. A more realistic attempt to assess errors on molecular clock estimates is required and this can be developed in hand with more rigorous assessments of the palaeontological data used in calibrating molecular clock analyses. However, unless these errors can be reduced, molecular clock estimates will remain of low practical value; the palaeontological record is imperfect but nevertheless provides the only firm constraint on the timing of clade divergence.

## Acknowledgements

Philippe Janvier (Museum National d'Histoire Naturelle, Paris) and Mike Coates (University of Chicago) provided useful reviews of the manuscript. Donoghue was funded through NERC Post Doctoral Research Fellowship GT5/99/ES/2; Smith was funded through NERC Research Grant NER/B/S/2000/00284, and Smith and Sansom were funded through NERC Research Grant GR3/10272.

## References

- Afanassieva, O.B. and Janvier, P. (1985) '*Tannuaspis*, *Tuwaspis* and *Ilemoraspis*, endemic osteostracan genera from the Silurian and Devonian of Tuva and Khakassia (USSR)', *Geobios*, 18: 493–506.

- Arduini, P., Pinna, G. and Teruzzi, G. (1981) 'Megaderaion sinemuriense n. g. n. sp., a new fossil enteropneust of the Sinemurian', *Atti della Società Italiana di Scienze Naturale e del Museo Civico di Storia Naturale di Milano*, 122: 104–8.
- Ayala, F.J., Rzhetsky, A. and Ayala, F.J. (1998) 'Origin of the metazoan phyla: molecular clocks confirm paleontological estimates', *Proceedings of the National Academy of Sciences, USA*, 95: 606–11.
- Bardack, D. (1991) 'First fossil hagfish (Myxinoidea): a record from the Pennsylvanian of Illinois', *Science*, 254: 701–3.
- (1998) 'Relationship of living and fossil hagfishes', in J.M. Jørgensen, J.P. Lomholt, R.E. Weber and H. Malte (eds) *The Biology of Hagfishes*, London: Chapman & Hall, pp. 3–14.
- Bardack, D. and Richardson Jr., E.S. (1977) 'New agnathous fishes from the Pennsylvanian of Illinois', *Fieldiana Geology*, 33: 489–510.
- Bardack, D. and Zangerl, R. (1968) 'First fossil lamprey: a record from the Pennsylvanian of Illinois', *Science*, 162: 1265–7.
- (1971) 'Lampreys in the fossil record', in M.W. Hardisty and I.C. Potter (eds) *The Biology of Lampreys*, London: Academic Press, pp. 67–84.
- Bengtson, S. and Urbanek, A. (1986) 'Rhabdotubus, a Middle Cambrian rhabdopleurid hemichordate', *Lethaia*, 19: 293–308.
- Benton, M.J. and Hitchin, R. (1996) 'Testing the quality of the fossil record by groups and habitats', *Historical Biology*, 12: 111–57.
- Benton, M.J. and Storrs, G.W. (1994) 'Testing the quality of the fossil record: paleontological knowledge is improving', *Geology*, 22: 111–14.
- Benton, M.J., Hitchin, R. and Wills, M.A. (1999) 'Assessing congruence between cladistic and stratigraphic data', *Systematic Biology*, 48: 581–96.
- Bleiweiss, R. (1998) 'Fossil gap analysis supports early Tertiary origin of trophically diverse avian orders', *Geology*, 26: 323–6.
- Blieck, A. (1984) 'Les Hétérostracés ptéraspidiformes, agnathes du Silurien-Dévonien du continent Nord-Atlantique et des Blocs Avoisnants: révision systématique, phylogénie, biostratigraphie, biogéographie', *Cahiers de Paléontologie, Centre national de la Recherche scientifique, Paris*, 1–199.
- Blieck, A. and Janvier, P. (1991) 'Silurian vertebrates', *Special Papers in Palaeontology*, 44: 345–89.
- Blieck, A., Elliot, D.K. and Gagnier, P.-Y. (1991) 'Some questions concerning the phylogenetic relationships of heterostracans, Ordovician to Devonian jawless vertebrates', in M.-M. Chang, Y.-H. Liu and G.-R. Zhang (eds) *Early Vertebrates and Related Problems in Evolutionary Biology*, Beijing: Science Press, pp. 1–17.
- Budd, G.E. and Jensen, S. (2000) 'A critical reappraisal of the fossil record of bilaterian phyla', *Biological Reviews*, 74: 253–95.
- Chen, J.-Y., Dzik, J., Edgecombe, G.D., Ramsköld, L. and Zhou, G.-Q. (1995) 'A possible Early Cambrian chordate', *Nature*, 377: 720–2.
- Chen, J.-Y., Huang, D.-Y. and Li, C.-W. (1999) 'An early Cambrian craniate-like chordate', *Nature*, 402: 518–22.
- Coates, M.I. (1994) 'The origin of vertebrate limbs', *Development*, 1994 Supplement: 169–80.
- Conway Morris, S. (1979) 'The Burgess Shale (Middle Cambrian) fauna', *Annual Review of Ecology and Systematics*, 10: 327–49.
- (1997) 'Molecular clocks: defusing the Cambrian explosion?' *Current Biology*, 7: R71–4.
- (2000) 'Evolution: bringing molecules into the fold', *Cell*, 100: 1–11.
- Cooper, R.A. (1999) 'The Ordovician time scale – calibration of graptolite and conodont zones', *Acta Universitatis Carolinae Geologica*, 43: 1–4.
- Cracraft, J. (2001) 'Avian evolution, Gondwana biogeography and the Cretaceous–Tertiary mass extinction event', *Proceedings of the Royal Society, London*, B268: 459–69.

- David, B. and Mooi, R. (1998) 'Major events in the evolution of echinoderms viewed by the light of embryology', in R. Mooi and M. Telford (eds) *Echinoderms: San Francisco*, Rotterdam: A.A. Balkema, pp. 21–8.
- (1999) 'Comprendre les échinodermes: la contribution du modèle extraxial-axial', *Bulletin de la Société géologique de France*, 170: 91–101.
- David, B., Lefebvre, B., Mooi, R. and Parsley, R. (2000) 'Are homalozoans echinoderms? An answer from the extraxial-axial theory', *Paleobiology*, 26: 529–55.
- Dayhoff, M.O. (1978) 'Survey of new data and computer methods of analysis', in M.O. Dayhoff (ed.) *Atlas of Protein Sequence and Structure*, Vol. 5, Supplement 3, Washington DC: National Biochemical Research Foundation, pp. 1–8.
- de Braga, M. and Reisz, R.R. (1995) 'A new diapsid reptile from the uppermost Carboniferous (Stephanian) of Kansas', *Palaeontology*, 38: 199–212.
- Delarbre, C., Barriel, V., Janvier, P. and Gachelin, G. (2002) 'Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly', *Molecular Phylogenetics and Evolution*, 22: 184–92.
- Donoghue, P.C.J., Forey, P.L. and Aldridge, R.J. (2000) 'Conodont affinity and chordate phylogeny', *Biological Reviews*, 75: 191–251.
- Donoghue, P.C.J. and Smith, M.P. (2001) 'The anatomy of *Turinia pagei* (Powrie) and the phylogenetic status of the Thelodonti', *Transactions of the Royal Society of Edinburgh (Earth Sciences)*, 92: 15–37.
- Durman, P.N. and Sennikov, N.V. (1993) 'A new rhabdopleurid hemichordate from the Middle Cambrian of Siberia', *Palaeontology*, 36: 283–96.
- Dzik, J. (1995) '*Yunnanzoon* and the ancestry of the vertebrates', *Acta Palaeontologica Polonica*, 40: 341–60.
- Encarnación, J., Rowell, A.J. and Grunow, A.M. (1999) 'A U–Pb age for the Cambrian Taylor Formation, Antarctica: implications for the Cambrian timescale', *Journal of Geology*, 107: 497–504.
- Feng, D.-F., Cho, G. and Doolittle, R.F. (1997) 'Determining divergence times with a protein clock: update and reevaluation', *Proceedings of the National Academy of Sciences, USA*, 94: 13028–33.
- Forey, P.L. (1984) 'Yet more reflections on agnathan–gnathostome relationships', *Journal of Vertebrate Paleontology*, 4: 330–43.
- Fortey, R.A., Briggs, D.E.G. and Wills, M.A. (1996) 'The Cambrian evolutionary "explosion": decoupling cladogenesis from morphological disparity', *Biological Journal of the Linnean Society*, 57: 13–33.
- (1997) 'The Cambrian evolutionary "explosion" recalibrated', *BioEssays*, 19: 429–34.
- Fredholm, D. (1988a) 'Vertebrate biostratigraphy of the Ludlovian Hemse Beds of Gotland, Sweden', *Geologiska Föreningens i Stockholm Förhandlingar*, 110: 237–53.
- (1988b) 'Vertebrates in the Ludlovian Hemse Beds of Gotland, Sweden', *Geologiska Föreningens i Stockholm Förhandlingar*, 110: 157–79.
- García-Fernández, J. and Holland, P.W.H. (1994) 'Archetypal organisation of the amphioxus *Hox* gene cluster', *Nature*, 370: 563–6.
- Gauthier, J., Kluge, A.G. and Rowe, T. (1988) 'Amniote phylogeny and the importance of fossils', *Cladistics*, 4: 105–209.
- Gee, H. (2001) 'Deuterostome phylogeny: the context for the origin and evolution of chordates', in P.E. Ahlberg (ed.) *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development*, London: Taylor & Francis, pp. 1–14.
- Gehling, J.G. (1987) 'Earliest known echinoderm – a new Ediacaran fossil from the Pound Subgroup of South Australia', *Alcheringa*, 11: 337–45.
- Glaessner, M.F. and Wade, M. (1966) 'The Late Precambrian fossils from Ediacara, South Australia', *Palaeontology*, 9: 599–628.

- Goodman, M., Miyamoto, M.M. and Czelisniak, J. (1987) 'Pattern and process in vertebrate phylogeny revealed by coevolution of molecules and morphologies', in C. Patterson (ed.) *Molecules and Morphology in Evolution: Conflict or Compromise?*, Cambridge: Cambridge University Press, pp. 141–76.
- Goujet, D. (2001) 'Placoderms and basal gnathostome apomorphies', in P.E. Ahlberg (ed.) *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development*, London: Taylor & Francis, pp. 209–22.
- Goujet, D. and Young, G.C. (1995) 'Interrelationships of placoderms revisited', *Geobios*, 19: 89–95.
- Gradstein, F.M. and Ogg, J. (1996) 'A Phanerozoic time scale', *Episodes*, 19: 3–5.
- Gross, W. (1969) '*Lophosteus superbus* Pander, ein Teleostome aus dem Silur Oesels', *Lethaia*, 2: 15–47.
- Hardisty, M.W. (1982) 'Lampreys and hagfishes: analysis of cyclostome relationships', in M.W. Hardisty and I.C. Potter (eds) *The Biology of Lampreys*, London: Academic Press, pp. 165–259.
- Hedges, S.B. (2001) 'Molecular evidence for the early history of living vertebrates', in P.E. Ahlberg (ed.) *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development*, London: Taylor & Francis, pp. 119–34.
- Hitchin, R. and Benton, M.J. (1996) 'Congruence between parsimony and stratigraphy: comparisons of three indices', *Paleobiology*, 23: 20–32.
- Holland, S.M. (1995) 'The stratigraphic distribution of fossils', *Paleobiology*, 21: 92–109.
- (2000) 'The quality of the fossil record: a sequence stratigraphic perspective', *Paleobiology*, 26 Supplement: 148–68.
- Hou, X., Ramsköld, L. and Bergström, J. (1991) 'Composition and preservation of the Chengjiang fauna – a Lower Cambrian soft-bodied biota', *Zoologica Scripta*, 20: 395–411.
- Huelsenbeck, J.P. (1994) 'Comparing the stratigraphic record to estimates of phylogeny', *Paleobiology*, 20: 470–83.
- Janvier, P. (1981) 'The phylogeny of the Craniata, with particular reference to the significance of fossil "agnathans"', *Journal of Vertebrate Paleontology*, 1: 121–59.
- (1996a) 'The dawn of the vertebrates: characters versus common ascent in the rise of current vertebrate phylogenies', *Palaeontology*, 39: 259–87.
- (1996b) *Early Vertebrates*, Oxford: Oxford University Press.
- (1998) 'Les vertébrés avant le Silurien', *Geobios*, 30: 931–50.
- (in press) 'Osteostraci', in H.-P. Schultze (ed.) *Handbook of Palaeoichthyology*.
- Janvier, P. and Blicek, A. (1993) 'L. B. Halstead and the heterostracan controversy', *Modern Geology*, 18: 89–105.
- Janvier, P. and Lund, R. (1983) '*Hardistiella montanensis* n.gen. et sp. (Petromyzontida) from the Lower Carboniferous of Montana, with remarks on the affinities of lampreys', *Journal of Vertebrate Paleontology*, 2: 407–13.
- Jefferies, R.P.S. (1967) 'Some fossil chordates with echinoderm affinities', *Zoological Society of London Symposium*, 20: 163–208.
- (1973) 'The Ordovician fossil *Lagynocystis pyramidalis* (Barrande) and the ancestry of amphioxus', *Philosophical Transactions of the Royal Society, London*, B265: 409–69.
- (1979) 'The origin of chordates: a methodological essay', in M.R. House (ed.) *The Origin of Major Invertebrate Groups*, London: Systematics Association, pp. 443–7.
- (1986) *The Ancestry of the Vertebrates*, London: British Museum (Natural History).
- Jefferies, R.P.S. and Lewis, D.N. (1978) 'The English Silurian fossil *Placocystites forbesianus* and the ancestry of the vertebrates', *Philosophical Transactions of the Royal Society, London*, B282: 205–323.
- Jefferies, R.P.S., Brown, N.A. and Daley, P.E.J. (1996) 'The early phylogeny of chordates and echinoderms and the origin of chordate left-right asymmetry and bilateral symmetry', *Acta Zoologica (Stockholm)*, 77: 101–22.

- Knoll, A.H. (2000) 'Learning to tell Neoproterozoic time', *Precambrian Research*, 100: 3–20.
- Kumar, S. and Hedges, S.B. (1998) 'A molecular timescale for vertebrate evolution', *Nature*, 392: 917–20.
- Kuraku, S., Hoshiyama, D., Katoh, K., Suga, K. and Miyata, T. (1999) 'Monophyly of lampreys and hagfishes supported by nuclear DNA-coded genes', *Journal of Molecular Evolution*, 49: 729–35.
- Lacalli, T.C. (2002) 'Vetulicolians – are they deuterostomes? chordates?' *BioEssays*, 24: 208–11.
- Lee, M.S.Y. (1999) 'Molecular clock calibrations and metazoan divergence dates', *Journal of Molecular Evolution*, 49: 385–91.
- Long, J.A. (1986) 'New ischnacanthid acanthodians from the Early Devonian of Australia, with comments on acanthodian interrelationships', *Zoological Journal of the Linnean Society* 87: 321–39.
- Løvtrup, S. (1977) *The Phylogeny of the Vertebrata*, New York: Wiley.
- Lund, R. and Janvier, P. (1986) 'A second lamprey from the Lower Carboniferous (Namurian) of Bear Gulch, Montana (U.S.A.)', *Geobios*, 19: 647–52.
- Mallatt, J. and Sullivan, J. (1998) '28S and 18S rDNA sequences support the monophyly of lampreys and hagfishes', *Molecular Biology and Evolution*, 15: 1706–18.
- Mallatt, J., Sullivan, J. and Winchell, C.J. (2001) 'The relationship of lampreys to hagfishes: a spectral analysis of ribosomal DNA sequences', in P.E. Ahlberg (ed.) *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development*, London: Taylor & Francis, pp. 106–18.
- Marshall, C.R. (1990) 'Confidence-intervals on stratigraphic ranges', *Paleobiology*, 16: 1–10.
- (1997) 'Confidence intervals on stratigraphic ranges with nonrandom distributions of fossil horizons', *Paleobiology*, 23: 165–73.
- Märss, T. (1986) 'Silurian vertebrates of Estonia and West Latvia', *Fossilia Baltica*, 1: 1–104.
- Modesto, S.P. (1995) 'The skull of the herbivorous synapsid *Edaphosaurus boanerges* from the Lower Permian of Texas', *Palaeontology*, 38: 213–39.
- Mooi, R. (2001) 'Not all written in stone: interdisciplinary syntheses in echinoderm paleontology', *Canadian Journal of Zoology*, 79: 1209–31.
- Mooi, R. and David, B. (1997) 'Skeletal homologies of echinoderms', *Paleontological Society Papers*, 3: 305–35.
- (1998) 'Evolution within a bizarre phylum: homologies of the first echinoderms', *American Zoologist*, 38: 965–74.
- Müller, K.J. (1977) '*Palaeobotryllus* from the Upper Cambrian of Nevada – a probable ascidian', *Lethaia*, 10: 107–18.
- Nei, M., Xu, P. and Glazko, G. (2001) 'Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms', *Proceedings of the National Academy of Sciences, USA*, 98: 2497–502.
- Nikoh, N., Iwabe, N., Kuma, K., Ohno, M., Sugiyama, T., Watanabe, Y., Yasui, K., Zhang, S., Hori, K., Shimura, Y. and Miyata, T. (1997) 'An estimate of divergence time of Parazoa and Eumetazoa and that of Cephalochordata and Vertebrata by Aldolase and Triose Phosphate Isomerase clocks', *Journal of Molecular Evolution*, 45: 97–106.
- Norell, M.A. (1992) 'Taxic origin and temporal diversity: the effect of phylogeny', in M.J. Novacek and Q.D. Wheeler (eds) *Extinction and Phylogeny*, New York: Columbia University Press, pp. 89–118.
- Norell, M.A. and Novacek, M.J. (1992) 'The fossil record and evolution: comparing cladistic and paleontologic evidence for vertebrate history', *Science*, 255: 1690–3.
- Novitskaya, L.I. (1971) *Les amphiaspides (Heterostraci) du Dévonien de la Sibérie*. Cahiers de Paléontologie, Centre national de la Recherche scientifique, Paris, 1–130.
- Paul, C.R.C. (1982) 'The adequacy of the fossil record', in K.A. Joysey and A.E. Friday (eds) *Problems of Phylogenetic Reconstruction*, London: Academic Press, pp. 75–117.

- (1998) 'Adequacy, completeness and the fossil record', in S.K. Donovan and C.R.C. Paul (eds) *The Adequacy of the Fossil Record*, Chichester: John Wiley & Sons, pp. 1–22.
- Paul, C.R.C. and Smith, A.B. (1984) 'The early radiation and phylogeny of echinoderms', *Biological Reviews*, 59: 443–81.
- Peterson, K.J. (1995) 'A phylogenetic test of the calcichordate scenario', *Lethaia*, 28: 25–38.
- Purnell, M.A. (2001) 'Scenarios, selection and the ecology of early vertebrates', in P.E. Ahlberg (ed.) *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development*, London: Taylor & Francis, pp. 187–208.
- Remane, J. (2000) 'International stratigraphic chart', International Union of Geological Sciences.
- Rodríguez-Trelles, F., Tarrío, R. and Ayala, F.J. (2002) 'A methodological bias toward overestimation of molecular evolutionary time scales', *Proceedings of the National Academy of Sciences, USA*, 99: 8112–15.
- Romer, A.S. (1933) 'Eurypterid influence on vertebrate history', *Science* 78: 114–17.
- Ruta, M. (1999) 'Brief review of the stylophoran debate', *Evolution & Development*, 1: 123–35.
- Sansom, I.J., Aldridge, R.J. and Smith, M.M. (2000) 'A microvertebrate fauna from the Llandovery of South China', *Transactions of the Royal Society of Edinburgh (Earth Sciences)*, 90: 255–72.
- Sansom, I.J., Smith, M.M. and Smith, M.P. (2001) 'The Ordovician radiation of vertebrates', in P.E. Ahlberg (ed.) *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development*, London: Taylor & Francis, pp. 156–71.
- Saylor, B.Z., Kaufman, A.J., Grotzinger, J.P. and Urban, F. (1998) 'A composite reference section for terminal Proterozoic strata of southern Namibia', *Journal of Sedimentary Research*, 68: 1223–35.
- Schultze, H.-P. (1968) 'Palaeoniscoidea-schuppen aus dem Unterdevon Australiens und Kansas und aus dem Mitteldevon Spitzbergens', *Bulletin of the British Museum (Natural History), Geology*, 16: 343–68.
- (1992) 'Early Devonian actinopterygians (Osteichthyes, Pisces) from Siberia', in E. Mark-Kurik (ed.) *Fossil Fishes as Living Animals*, Tallinn: Academy of Sciences of Estonia, pp. 233–42.
- Shu, D.-G., Zhang, X. and Chen, L. (1996a) 'Reinterpretation of *Yunnanozoon* as the earliest known hemichordate', *Nature*, 380: 428–30.
- Shu, D.-G., Conway Morris, S. and Zhang, X.-L. (1996b) 'A *Pikaia*-like chordate from the Lower Cambrian of China', *Nature*, 384: 157–8.
- Shu, D.-G., Luo, H.-L., Conway Morris, S., Zhang, X.-L., Hu, S.-X., Chen, L., Han, J., Zhu, M., Li, Y. and Chen, L.-Z. (1999) 'Lower Cambrian vertebrates from south China', *Nature*, 402: 42–6.
- Shu, D.-G., Chen, L., Han, J. and Zhang, X.-L. (2001a) 'An early Cambrian tunicate from China', *Nature*, 411: 472–3.
- Shu, D.-G., Conway Morris, S., Han, J., Chen, L., Zhang, X.-L., Zhang, Z.-F., Liu, H.-Q., Li, Y. and Liu, J.-N. (2001b) 'Primitive deuterostomes from the Chengjiang Lagerstätte (Lower Cambrian, China)', *Nature*, 414: 419–24.
- Siddall, M.E. (1996) 'Stratigraphic consistency and the shape of things', *Systematic Biology*, 45: 111–15.
- (1997) 'Stratigraphic indices in the balance: a reply to Hitchin and Benton', *Systematic Biology*, 46: 569–73.
- (1998) 'Stratigraphic fit to phylogenetics: a proposed solution', *Cladistics*, 14: 201–8.
- Smith, A.B. (1984) 'Classification of the Echinodermata', *Palaeontology*, 27: 431–59.
- (1988a) 'Patterns of diversification and extinction in Early Palaeozoic echinoderms', *Palaeontology*, 31: 799–828.

- Smith, A.B. (1988b) 'Fossil evidence for the relationships of extant echinoderm classes and their times of divergence', in C.R.C. Paul and A.B. Smith (eds) *Echinoderm Phylogeny and Evolutionary Biology*, Oxford: Clarendon Press, pp. 85–97.
- (1990) 'Evolutionary diversification of echinoderms during the early Palaeozoic', in P.D. Taylor and G.P. Larwood (eds) *Major Evolutionary Radiations. Systematics Association Special Publication No. 42*, Oxford: Clarendon Press, pp. 265–86.
- (1999) 'Dating the origin of metazoan body plans', *Evolution & Development*, 1: 138–42.
- (2001) 'Large-scale heterogeneity of the fossil record: implications for Phanerozoic biodiversity studies', *Philosophical Transactions of the Royal Society, London*, B356: 351–67.
- Smith, A.B. and Peterson, K.J. (2002) 'Dating the time of origin of major clades: molecular clocks and the fossil record', *Annual Review of Earth and Planetary Science*, 30: 65–88.
- Smith, A.B., Gale, A.S. and Monks, N.E.A. (2001) 'Sea level change and rock bias in the Cretaceous: a problem for extinction and biodiversity studies', *Paleobiology*, 27: 241–53.
- Smith, M.M. and Sansom, I.J. (1997) 'Exoskeletal microremains of an Ordovician fish from the Harding Sandstone of Colorado', *Palaeontology*, 40: 645–58.
- Smith, M.P., Sansom, I.J. and Cochrane, K.D. (2001) 'The Cambrian origin of vertebrates', in P.E. Ahlberg (ed.) *Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development*, London: Taylor & Francis, pp. 67–84.
- Smith, M.P., Donoghue, P.C.J. and Sansom, I.J. (2002) 'The spatial and temporal diversification of Early Palaeozoic vertebrates', in J.A. Crame and A.W. Owen (eds) *Palaeobiogeography and Biodiversity Change: the Ordovician and Mesozoic–Cenozoic Radiations*, Geological Society Special Publication 194: 69–83.
- Springer, M.S. (1997) 'Molecular clocks and the timing of the placental and marsupial radiations in relation to the Cretaceous–Tertiary boundary', *Journal of Mammalian Evolution*, 4: 285–302.
- Stensiö, E.A. (1927) 'The Downtonian and Devonian vertebrates of Spitsbergen. Part 1. Family Cephalaspidae', *Skrifter om Svalbard og Nordishavet*, 12: 1–391.
- (1968) 'The cyclostomes, with special reference to the diphyletic origin of the Petromyzontida and Myxinoidea', in T. Ørvig (ed.) *Current Problems in Lower Vertebrate Phylogeny, Nobel Symposium 4*, Stockholm: Almquist & Wiksell, pp. 13–71.
- Stock, D.W. and Whitt, G.S. (1992) 'Evidence from 18S ribosomal RNA sequences that lampreys and hagfishes form a natural group', *Science*, 257: 787–9.
- Strauss, D. and Sadler, P.M. (1989) 'Classical confidence-intervals and Bayesian probability estimates for ends of local taxon ranges', *Mathematical Geology*, 21: 411–21.
- Sumrall, C.D. (1997) 'The role of fossils in the phylogenetic reconstruction of the Echinodermata', *Paleontological Society Papers*, 3: 267–88.
- Suzuki, M., Kubokawa, K., Nagasawa, H. and Urano, A. (1995) 'Sequence analysis of vasotocin cDNAs of the lamprey *Lampetra japonica*, and the hagfish, *Eptatretus burgeri*: evolution of cyclostome vasotocin precursors', *Journal of Molecular Endocrinology*, 14: 67–77.
- Sweet, W.C. (1988) *The Conodonts: Morphology, Taxonomy, Paleoecology, and Evolutionary History of a Long-extinct Animal Phylum*, Oxford: Clarendon Press.
- Sweet, W.C. and Donoghue, P.C.J. (2001) 'Conodonts: past, present and future', *Journal of Paleontology*, 75: 1174–84.
- Tavaré, S., Marshall, C.R., Will, O., Soligo, C. and Martin, R.D. (2002) 'Using the fossil record to estimate the age of the last common ancestor of extant primates', *Nature*, 416: 726–9.
- Taverne, L. (1997) '*Osorioichthys marginis*, "paleonisciform" from the Fammenian of Belgium, and the phylogeny of the Devonian actinopterygians (Pisces)', *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique*, 67: 57–78.

- Tucker, R.D. and McKerrow, W.S. (1995) 'Early Paleozoic chronology: a review in light of new U-Pb zircon ages from Newfoundland and Britain', *Canadian Journal of Earth Sciences*, 32: 368–79.
- Tucker, R.D., Bradley, D.C., Ver Straeten, C.A., Harris, A.G., Ebert, J.R. and McCutcheon, S.R. (1998) 'New U-Pb zircon ages and the duration and division of Devonian time', *Earth and Planetary Science Letters*, 158: 175–86.
- Ubahgs, G. (1968) 'Stylophora', in R.C. Moore (ed.) *Treatise on Invertebrate Paleontology. Part 5. Echinodermata 1(2)*, Boulder and Lawrence KS: Geological Society of America and University of Kansas Press, pp. 496–565.
- van Tuinen, M., Sibley, C.G. and Hedges, S.B. (1998) 'Phylogeny and biogeography of ratite birds inferred from DNA sequences of the mitochondrial ribosomal genes', *Molecular Biology and Evolution*, 15: 370–6.
- Wagner, P.J. (1998) 'Phylogenetic analysis and the quality of the fossil record', in S.K. Donovan and C.R.C. Paul (eds) *The Adequacy of the Fossil Record*, Chichester: John Wiley & Sons Ltd, pp. 165–87.
- Wang, D.Y.C., Kumar, S. and Hedges, S.B. (1998) 'Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi', *Proceedings of the Royal Society, London*, B266: 163–71.
- Weiss, R.E. and Marshall, C.R. (1999) 'The uncertainty in the true end point of a fossil's stratigraphic range when stratigraphic sections are sampled discretely', *Mathematical Geology*, 31: 435–53.
- Wills, M.A. (1999) 'Congruence between phylogeny and stratigraphy: randomization tests and the gap excess ratio', *Systematic Biology*, 48: 559–80.
- Wills, M.A. and Fortey, R.A. (2000) 'The shape of life: how much is written in stone?' *BioEssays*, 22: 1142–52.
- Wills, M.A. and Sepkoski, J.J. (1993) 'Problematica', in M.J. Benton (ed.) *The Fossil Record 2*, London: Chapman & Hall, pp. 543–54.
- Wray, G.A., Levinton, J.S. and Shapiro, L.H. (1996) 'Molecular evidence for deep Precambrian divergences among metazoan phyla', *Science*, 274: 568–73.
- Yalden, D.W. (1985) 'Feeding mechanisms as evidence of cyclostome monophyly', *Zoological Journal of the Linnean Society*, 84: 291–300.
- Young, G.C. (1991) 'The first armoured agnathan vertebrates from the Devonian of Australia', in M.M. Chang, Y.H. Liu and G.R. Zhang (eds) *Early Vertebrates and Related Problems in Evolutionary Biology*, Beijing: Science Press, pp. 67–85.
- Zhu, M. and Schultze, H.-P. (1997) 'The oldest sarcopterygian fish', *Lethaia*, 30: 293–304.
- Zrzavý, J., Mihulka, S., Kepka, P., Bezdek, A. and Tietz, D. (1998) 'Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence', *Cladistics*, 14: 249–85.
- Zuckerandl, E. and Pauling, L. (1962) 'Molecular disease, evolution and genic heterogeneity', in M. Kasha and B. Pullman (eds) *Horizons in Biochemistry*, New York: Academic Press, pp. 189–225.
- (1965) 'Evolutionary divergence and convergence in proteins', in V. Bryson and H.J. Vogel (eds) *Evolving Genes and Proteins*, New York: Academic Press, pp. 97–166.

## Appendix 10.1

Codings for taxa augmented to the analysis of Donoghue and Smith (2001):

*Myllokunmingia*

0????????????????????????????????1?10????0????????10010?0????0????000000000000000000?0?????  
 ?????????????????000?001

*Haikouichthys*

0????????????????1????????10????0????????110?0???0????000000000000000000?0???????  
 ?????????????000?001



## Appendix 10.2

Tree topologies and occurrence data upon which cladogram–stratigraphy statistics and confidence intervals are based.

### Conodonta

((*Proconodontus* ((Fryxellodontidae Pygodontidae)(Cordylodontidae (Ansellidae (Dapsilodontidae Belodellidae)))))(*Teridontus* ((Clavohamulidae (Drepanoistontidae (Acanthodontidae Panderodontidae)))(*Rossodus* (Multioistodontidae (Periodontidae (Rhipidognathidae (Prioniodontidae ((Cyrtoniodontidae ((Chirognathidae Prioniodinidae) Kockelellidae))(Polyplacognathidae (Distomodontidae (Icriodellidae Icriodontidae)))))))))); following Sweet & Donoghue (2001), after Sweet (1988).

### Heterostracomorpha

(Astraspida (Arandaspididae (Lepidaspididae Tesseractidae Phialaspidiformes (Corvaspids/Tolypepids ((Cyathaspidida (Ctenaspididae ((Eglonaspididae Hibernaspididae) (Siberiaspididae (Amphiaspididae Olbiaspididae)))))) Anchipteraspididae (Protopteraspididae (Pteraspidae (Protaspidae Psammosteidae)))))); following Novitskaya (1971), Blicek (1984) and Janvier (1996b).

### Anaspida

(*Pharyngolepis*,(*Pterygolepis*,(*Rhyncholepis*,*Lasanius*,*Birkenia*))); following Janvier (1996b).

### Thelodontii

(Furcacaudiformes (Thelodontids (Loganellidae Phlebolepidae))); following Donoghue & Smith (2001).

### Galeaspida

(Dayongaspididae Hanyangaspis Xiushuiaspididae (((((Eugaleaspidae Tridensaspidae) *Nochelaspis*) *Yunnanogaleaspis*) Sinogaleaspidae) ((((*Duyunolepis* *Neoduyunaspis* *Paraduyunaspis*) *Dongfangaspis* Polybranchiaspidae) *Bannhuanaspis*) Huananaspidida)); following Janvier (1996b).

### Osteostraci

(*Ateleaspis* (*Aceraspis* (*Hirella* (*Hemiteleaspis* (*Hemicyclaspis* (Escuminaspididae Tannuaspididae (Cephalaspididae (Mimetaspididae Pattenaspididae)) (Zenaspida ((*Tauraspis* (*Hapilaspis* Benneviaspididae (Hoelaspididae Boreaspididae))) ((*Procephalaspis* (*Auchenaspis* (*Witaaspis* (*Thyestes* Tremataspididae))) (*Kiaeraspis* (Axinaspididae Acrotomaspididae)))))))))); following Janvier (1996b; in press)

### Chondrichthyes

(Cladoselachidae (Eugeneodontida Petalodontida) (Inopterygia ((Helodontidae (Cochliodontidae (Echinochimeridae Chimeridae))) (Symmoriidae Stethacanthidae)) (Xenacanthiformes (*Ctenacanthus* (Hybodontiformes Neoselachii))); following Janvier (1996b).

### Placodermi

((((Actinolepida (Phyllolepida Wuttagoonaspida)) (Phlyctaenii (*Gemeundaspis* (Holonematidae ((Homostiidae Buchanosteidae) ((Brachydeiroidea (Coccosteidae Camuropiscidae)) (Dinichthyidae Aspinothoraci)))))) (Ptycyodontida Petalichthyida))

((Yunnanolepididae (Sinolepidae ((Bothriolepidae Microbrachiidae) (Pterichthyoidea Asterolepidae)))) Rhenanida)); following Goujet & Young (1995) and Janvier (1996b).

#### **Acanthodii**

(Ischancanthidae (((Climatiidae Gyranthidae) (Diplacanthidae Culmacanthidae)) (Mesacanthidae (Cheiracanthidae Acanthodidae)))); following Long (1986).

#### **Actinopterygii**

(*Lophosteus* (*Andreolepis* (*Naxilepis* (*Orvikuina* (*Ligulalepis* (*Dialipina* (*Cheirolepis* (*Polypterus* (*Osorioichthys* (*Howqualepis* (*Mimia* (*Moythomasia* (*Tegeolepis* (*Stegotrachelus* (*Kentuckia*)))))))))))); following Schultze (1992) and Taverne (1997).