the TIMETREE of LIFE

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Calibrating and constraining molecular clocks

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Abstract

In dating phylogenetic trees, it is important to work to the strengths of paleontology and molecular phylogeny estimation. Minimum constraints on calibrations (i.e., oldest fossils in a crown clade) may be calculated with some precision and may be treated as hard bounds, while maximum constraints are soft bounds that may be represented most honestly by probability distributions that reflect the distribution of fossiliferous rocks around the time in question, but allow a small probability of truly ancient dates as well. We present detailed documentation of 63 key calibration dates, with thorough evidence and error expressions, for a wide range of organisms.

For well over 200 years, natural scientists have used fossils with varying degrees of confidence to date the evolution of life. The field has advanced dramatically in the last few years, and it would be useful now to review some of the key issues and to suggest an outline of a *modus operandi* for the future.

In explaining the role of fossils in establishing timescales, it is useful to review the historical sequence in which key observations were made. Much of this early history predates the 1960s concept of the molecular clock; but the way in which fossils should be used today depends crucially on those earlier geological and paleontological observations. In presenting these observations in a logical sequence, we highlight what can and cannot be done with fossils, and link this to the relative strengths and weaknesses of molecular data. The value of carrying out this survey now is that it is not framed in the old and rather worn narrative of a "conflict" between fossils and molecules (e.g., *1, 2*). It is no longer a question of which is better than the other, or how far one can go with one or the other source of data. It is evident that both fossils and molecules have great strengths, but in recognizing the weaknesses of each source of data, a realistic plan for collaboration between paleontologists and molecular biologists can be proposed (*3, 4*).

In this chapter we review the key qualities of the fossil record in a semi-historical account that provides explanations and key references. We then outline a framework for collaboration in dating the tree of life. Finally, we present further, documented, evidence of key fossil-based data for dating.

The key attributes of the fossil record

Fossils occur in temporal order

It seems self-evident that older rocks lie at the bottom of the pile, and younger rocks in successive layers on top. And yet, it was only when Nicholas Steno (1638–1686) in 1669 enunciated this as the law of superposition of strata (*5*) that observers took the point. Fossils were known in Steno's day, but they were seen as rather random in occurrence and not linked to any pattern reflecting the history of life. By the 1790s, when scientists had finally accepted the idea of extinction, Georges Cuvier (1769–1832) in France and William Smith (1769–1839) in England showed that assemblages of fossils occur in successive rock strata and that the sequence of rocks and of fossils reflects some aspects of the history of the Earth and of life. In more recent times, this conclusion has been confirmed by the observation that temporally older vertebrate fossils tend to occupy cladistically more basal nodes (cf. *6*).

Fossils and fossil assemblages are characteristic of units of past time

Cuvier and Smith also noted the predictability of some aspects of the fossil record, in particular the totality of fossils that had been collected. Specifically, they noted that particular fossils, or assemblages of fossils, appeared

to characterize particular rock units and to occur in the same order every time they were seen. This observation was used practically by geologists to correlate rock units from place to place, to name them (e.g., "Carboniferous," "Lias," "Old Red Sandstone") and to match stratigraphic units on the first geological maps. The principle is fundamental to modern biostratigraphy and lies behind the practical search for new sources of oil and gas today. However, the practices of biostratigraphy and correlation have evolved substantially, in particular with the development of graphic correlation which facilitates the integration of stratigraphic phenomena, including isotope anomalies and geochronologically dated ash layers (7), to derive a global composite standard stratigraphy against which local sections may be compared and correlated (8).

Fossils may be included in phylogenies of modern organisms

Cuvier had no sympathy with then-current ideas of evolution, but he gave the world the science of comparative anatomy. Whatever the causes, he recognized the prevalence of anatomical similarities in often widely different organisms, and he realized these indicated closeness of relationship in some sense. He famously demonstrated his skills in public demonstrations in Paris in the early nineteenth century, by taking a single fossil bone, and reconstructing the whole animal and its biology and habits in some detail, before an assistant revealed the whole skeleton. Cuvier never plotted an evolutionary tree, but key concepts such as homology arose from his pre-Darwinian comparative anatomy.

There is a single geological timescale that may be used as a yardstick of time

William Smith around 1800 mapped his correlatable geological units across England, and guessed they extended over Europe. Roderick Murchison (1792–1871) and others in the 1830s drove these ideas forward, naming the major divisions of geological time, the Paleozoic, Mesozoic, and Cenozoic eras, and the various geological periods (Cambrian, Silurian, Devonian, etc.). Murchison hurtled across Europe and Russia in his coach and mapped his British units across the Ural Mountains. He declared that the new geological eras and periods, established in Western Europe (mainly in England and Wales), provided a yardstick of deep time that would work worldwide. Initial studies in Africa, North America, and

Australia about 1840 showed he was right. Since 1840, the international geologic timescale has not been substantially revised, but some major time units have been added, most notably the Ediacaran in 2004 (9).

The order of fossils matches the pattern of the evolution of life

Although faintly discerned by Smith, Cuvier, and others, the link between deep time and the phylogeny of life could not be made without an understanding that life had evolved. Cuvier's great rival at the Muséum Nationale d'Histoire Naturelle in the 1790s, Jean-Baptiste Lamarck (1744–1829), was the first serious proponent of evolution, the idea that species change through time. His model of evolution was more an escalator than a tree, the great *scala naturae*, where every species is arrayed along one or more moving walkways from rocks to angels: the humans of today were once apes, and the apes of today may some day be humans. Many people in the 1830s accepted the idea of progress, or directionality, in the order of fossils in the rocks, but others, notably Charles Lyell (1797–1875), saw time as a series of cycles rather than a unidirectional arrow, and so sought to deny the idea of progress from simple to complex through the succession of fossils in the rocks.

There is a tree of life

Charles Darwin (1809–1882) was the first to understand that the evolution of life was not an escalator, nor any other kind of linear progression, but a branching tree that links all living and fossil species, and the lines unite backwards in time to the single common ancestor of all life. His first branching tree appeared in manuscript notes in 1838; and the sole illustration in the *Origin of Species* was a branching tree (10).

The fossil record is incomplete

Charles Darwin also spent some time in the *Origin of Species* discussing the "imperfection of the geological record." He pointed out that many living organisms have only soft parts, and so are unlikely to be preserved. Others live in environments such as mountainsides or beaches, where erosion dominates, and sediment does not accumulate. He noted the patchiness of geological strata, and the fact that intermediate fossil forms are rare. Darwin did, however, predict that intense efforts by paleontologists would fill many of the gaps in the

record and allow the deeper parts of the tree of life to be disentangled. In many cases (e.g., basal tetrapods, synapsids, and diapsids) his prediction has been fantastically confirmed, whereas others remain more difficult to document paleontologically. Raup (*11*) summarized the issue clearly, arguing that there are biases in the fossil record; for example, the quality of the record must diminish as one goes further back in time. It has been shown, however, that although there is a diminution in quality back in time, this does not erase large-scale evolutionary patterns (*12*).

The fossil record can only be as complete as the rock record in which it is preserved; and since attempts have been made to compile large-scale databases of organismal diversity though geological time, there has been a worry that it is biased by inconsistencies in the rock record (*11*). These include the consequence of plate tectonics, like the fact that open ocean sediments are invariably destroyed at destructive plate margins, along with the oceanic crust on which they rest. Thus, the only open ocean sediments from which we may sample past diversity are of Triassic age or younger; older sediments from these environments are very rare and are represented only by tectonically and thermally abused mélanges scraped from the surface of oceanic crust as it subducted into the Earth's mantle. Continental interiors are invariably regions of net erosion, rather than sedimentation, and so it is only at the continental margins that we can hope to maintain a record of relatively continuous sedimentation, in which fossil remains may be preserved over long geological timescales. However, because environments shift in position with respect to the rise and fall of sea level over geological timescales, there are concomitant secular variations in the environments—and their hosted organisms—that are preserved (*13*).

Most worrying of all, variance in the availability of rock for sampling and the numbers of species found in those rocks are closely correlated (*14–16*). This may indicate either that biases in the rock record dominate the paleontological diversity signal, or that the rise and fall in sea level controls rock volume and species diversity. These alternative hypotheses are difficult to reconcile (*17*). However, the general congruence between phylogenetic branching order and the temporal sequence in which fossils are found (*6, 12*) indicates that even if the rock record is heterogeneous, the primary paleontological signal is not overwhelmed. Thus, we may place some faith in the fossil record, our only direct record of evolutionary history, but it must nevertheless be interpreted with considerable care.

Deep time may be dated

Throughout the nineteenth century, most scientists accepted that the Earth was very ancient, but there was no meaningful way to determine exact dates for any rocks. Calculations based on estimates of the rate of cooling of the Earth from an initial supposedly molten state gave rise to rather short histories of the Earth, measured in tens or at best hundreds of millions of years, whereas estimates based on rates of sedimentation or the rate of solution of salts in the sea, yielded rather higher estimates, in the hundreds to thousands of millions of years. The discovery and application of radiometric dating by Arthur Holmes (1890–1965) and others in the early years of the twentieth century, showed that the Earth was some 4.5 billion years old, and that the fossiliferous Phanerozoic represented the past 0.5 billion years or so (*18*). Further details of radiometric dating are given in The Geologic Time Scale. Radiometric dating has evolved substantially and, although the geologic timescale is in constant revision, there is an ever-diminishing error, and the calibration of geologic time is being extended into the Proterozoic (*19*).

A dated phylogenetic tree offers valuable information on evolution

In the early to mid-twentieth century, various paleontologists used trees of one sort or another to calculate rates of evolution, whether rates of change of individual characters, of all the characters of a group of organisms (the transformational approach), or rates of appearance and turnover of species or genera (the taxic approach). Even with uncertain timescales, such rates could be established in a relative way, as shown by George Gaylord Simpson (1902–1984) in his classic work, *Tempo and Mode in Evolution* (*20*). Since the 1940s, there has been a renaissance in paleobiology, with extensive work on rates and patterns of evolution, origination and extinction. Effective molecular clock methodologies afford a new opportunity for both molecular and morphological evolution, allowing us to approach fundamental questions such as the relationship between morphological and molecular evolution (*21, 22*).

Phylogenies are cladistic

Until the 1960s, phylogenies were put together in a somewhat impressionistic way, whereby systematists used their judgment to determine propinquity of relationship. There was a concept of phylogenetically useful morphological

characters, as opposed to less useful characters, but there were no clear rules or protocols that allowed analyses to be repeated or challenged. Hennig (*23*) famously distinguished plesiomorphic ("ancestral") from apomorphic ("derived") characters, but it is well known also that his message was not widely appreciated until his book was translated into English in 1966 (*24*).

Fossils may belong to stems or crowns

There has been much confusion in discussions about the tree of life because of sloppy use of taxic terms. Hennig (*24, 25*) and Jefferies (*26*) distinguished crowns and stems: a crown clade consists of all living members of a group, their common ancestor, and everything in between, regardless of whether it is living or extinct. A stem is paraphyletic and composed of all those extinct lineages more closely related to the crown group in question, than to another (Fig. 1). So *Archaeopteryx* is a stem bird but not a crown bird. This confusion between stems and crowns led to some difficulty in the early debates about the timing of origin of major clades (*27, 28*) but while clarification of these issues has led to the resolution of dispute in some debates (*29*), it has actually increased confusion in others (*30, 31*). Fossil taxa need not exhibit all derived characters of the crown clade in question because, invariably, we seek a date to constrain divergence between one crown group and another. Thus, it is the fossils assigned to the stem that, by definition, lack the full complement of crown-group characters, in which we have most interest. However, fossils may be fragmentary and it may be difficult to distinguish whether the absence of characters in a fossil taxon reflects an aspect of evolution or simply incomplete preservation.
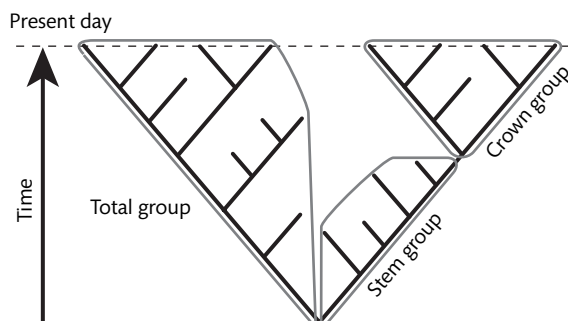

Fig. 1 Stem, crown and total-group definitions, following Hennig (*25*) and Jefferies (*26*).

With an understanding of the strengths and limitations of fossils, it will now be possible to consider current assumptions about how fossils may be used to contribute to the grand enterprise of dating the tree of life.

Strategies for using fossils to date trees

There are many practitioners of tree dating, and each has a more-or-less unique approach to the problem. The emphasis on fossils as useful (or not), the number of calibration points recommended, the ways in which fossil-based dates should be determined and cited, the ways in which such calibration dates should be assessed for congruence or not, and how they should be combined with molecular trees, are all under active debate. We consider these points in turn, and seek to make broadly defensible recommendations that may contribute to the development of a new protocol that will be generally acceptable. We are optimistic that a fair number of previously debated points may now be resolved, and that methods now exist that play to the strengths of paleontological data on the one hand, and molecular data on the other (*3, 4*).

One calibration date or many?

It was commonplace through the 1960s to 1990s to use a single calibration point in molecular clock analyses, generally because data sets were small and algorithms simpler. Most analysts of tetrapod phylogeny, for example, used the mammal–bird date of 305–315 Mya (*32*) as their sole reference point. This stance has been criticized (*33–37*) because of the risk that the whole enterprise will be skewed by possible errors in the sole calibration point. Others (e.g., *35, 38–40*) have argued forcefully that multiple calibration dates should be used, suggesting that greater numbers will reduce uncertainty and improve statistical robustness. It is perhaps generally correct that several dates are better than one, but it would be facile to argue that more is always better. There is no benefit in simply increasing the number of paleontological dates used in a calibration exercise without thought about their quality: numerous erroneous dates will give a meaningless result (*41–43*).

Cross validation of potential calibration points

One problem with using multiple fossil calibration dates is that there is a risk of mixing useful and erroneous calibrations. Near and Sanderson (*42*) and Near *et al.* (*41*)

have suggested that it is better to cross validate different potential calibration points across a single phylogeny and determine which are consistent with each other, and which are inconsistent. The consistent calibration dates, which all point to the same solutions for unknown dates are assumed to be a more-or-less correct set of fossil dates, close to their relevant nodes. The inconsistent dates may be too young or too old, indicating either unusually poorly sampled lineages (too young) or incorrectly assigned fossils (too old). Although it is likely that this method will indeed identify a consistent set of dates in most cases, and these dates will presumably be close to the relevant nodes, this need not be the case. We note that cross validation methods may not always work: Hugall *et al.* (*44*) note a case where two calibration points can be correct but appear incongruent because rate smoothing failed to give the correct relative branch lengths.

In an instructive application of cross validation of paleontological calibration dates, Douzery *et al.* (*45*) applied seven minimum divergence estimates from the mammalian fossil record to their molecular clock study focusing on rodents. Four were within crown Rodentia, one based on a close relative of crown Lagomorpha, one from crown Cetartiodactyla, and one based on a 1996 report of a "Paleocene" proboscidean taxon (*Phosphatherium*) from Morocco (*46*). When analyzed alone, each of these calibrations predicted at least one of the six other dates within a 95% confidence interval except for one: their Paleocene calibration for Paenungulata. Importantly, it was exactly this date that had been geologically misinterpreted in its original publication (*46*). According to more recent analyses of the Moroccan Ouled Abdoun Basin localities from which *Phosphatherium* is known (*47*), the original report of a Paleocene age was in error; these fossils are not Paleocene but Eocene, ca. 5–7 Ma younger than the 60 Ma value given in Douzery *et al.* (*45*). The early Eocene remains of proboscideans from Morocco remain among the oldest known fossils of crown Afrotheria. Although somewhat by accident, Douzery *et al.* (*45*) recognized this very instructive error by identifying their 60 Ma paenungulate date as incompatible with any of their other six calibration points. Importantly, the resulting clock estimates from Douzery *et al.* (*45*) for placental mammals yielded dates within the Tertiary for intraordinal divergences, and did not exceed 80 Ma for the common ancestor of Placentalia. These values are generally consistent with estimates based on the mammalian fossil record (*48*) and with some other, independent molecular clock estimates (*49*), but not others (e.g., *50–52*).

It is possible that cross validating paleontological minima may make the resulting estimates "too young." Such minimum estimates from the fossil record are, after all, "minimum" and, barring misidentification, they will underestimate actual divergence times. However, when several such calibrations are mutually compatible (which is distinct from biasing dates in a single direction), this indicates either accurate identification of a genuine divergence date or systematic error affecting all calibrations, as may be the case when preservational factors affect multiple fossil lineages. Such factors usually act in limited geographic areas, and fossil finds from other regions can plug gaps. Some geologic events, such as major sea-level changes, can create a global-scale hiatus, such as the nonpreservation of coastal habitats. Such global hiatuses are generally brief, geologically speaking, and the habitat and its fossil record reappear, and so can be identified and measured. In any event, all such cases must be evaluated individually and on their own merits. In general, we regard cross validation as a valuable tool to help improve the accuracy of fossil calibrations as applied in molecular clock studies.

Choice of dates and the quality of the fossil record

Fossil date estimates for divergence events have errors associated with them that arise because of all the various imperfections of the fossil record, as well as the often tortuous means by which a numerical date can be assigned. However, these errors are rarely if ever acknowledged—an astonishing fact given that calibration is, by definition, the rate-determining step in molecular clock analyses. Reisz and Müller (*37, 53–55*) have argued that this may be overcome by quoting errors on the dates, where the errors are indicated by the age of more and less derived relatives of the fossil organism that provides the main calibration date. Thus, an age span, rather than a point date can be used to calibrate a clock, faithfully reflecting the error associated with the paleontological estimate.

However, Reisz and Müller go further and argue that some paleontological estimates have much broader errors than others, providing a measure of their relative quality. In particular, they single out the bird–mammal split, which is the most widely adopted of all fossil calibrations, as a less-than-ideal example of paleontological calibration. This is because of a dearth of more primitive relatives that are close in age; indeed, there is a dearth of sites from which such fossils might be found. Thus, the errors on the paleontological estimate, particularly for its

maximum bound, are very broad indeed. Many alternative calibrations are available with much smaller attendant errors associated with them. For some questions, it may still be desirable to use the bird–mammal split as a calibration, because of its applicability to a breadth of sequence data in public databases and relevance to certain high-profile scientific questions. Nevertheless, Reisz and Müller have made a valid point with which we fundamentally agree: not all calibrations are of the same quality and when possible, those with more paleontological data pertinent to constraining a soft maximum estimate (as when fossil-bearing strata older than the minimum estimate are well sampled) should be preferred.

The debate about accuracy of paleontological calibration dates conflates two issues: the relative accuracy of minimum and maximum constraints. For the minimum constraint, the dating error on a securely identified fossil is simply the error in dating the rock formation in which it is contained. For a maximum constraint, the error encompasses this error but, much more significantly, it is also a measure of uncertainty that the oldest possible age estimate really lies below the branching point in question. In the next sections, we argue first that error on a fossil calibration cannot be symmetrical and cannot be generalized about a single fossil point. We then show that fossils can act as relatively secure "hard" minimum constraints on a particular branching point, and a soft maximum constraint can also be estimated.

Magnitude and symmetry of error on fossil calibration dates

Error bars on paleontological calibration points, if used at all, have generally been assumed to be symmetrical. This seems logical, because uncertainties about dating rock layers and uncertainties about the identity of the fossil might be assumed to be equal in both directions, up and down. However, errors were usually not indicated on fossil calibration dates because there has not been an obvious or reasonable way to calculate them (3).

One "quick fix" for this problem was proposed by Douzery et al. (40), who used the whole span of the geological period in which the calibration fossil was found as their error range. So, for example, they used the span of the Devonian period (354–417 myr) as the error on timing of the split of mammals and actinopterygian fishes (i.e., the base of clade Osteichthyes). As Hedges et al. (56) pointed out, this is a far wider age range than is necessary when compared to either the date of the oldest osteichthyan (418.7 ± 2.6 myr), or the range from soft

maximum to minimum constraint for that branching point (421.75–416 myr).

The debate about error bars on fossil dates, whether to use them or not, how to calculate them, and whether they are symmetrical or not, is circumvented, we suggest, by a recognition of the use of fossils to determine minimum and soft maximum constraints.

Fossil dates as estimates of origin or as minimum constraints

Until recently, paleontologists and molecular clock practitioners have been perhaps a little unclear about just what the fossil dates represent. The tenor of many of the to-and-fro debates between defenders and opponents of the merits of the fossil record in dating the tree of life (e.g., 1, 2) suggested that both sides were treating their dates as pointing at the same thing, namely the actual time of origin of a clade. It is clear, however, that the two dates are different. Paleontologists are limited in recognizing the origin of a clade because clades may start as rare, founding taxa, located in only one small part of the world and, by definition, they will lack many or all diagnostic crown-group characters. So, the oldest fossil X will always be younger than the origin of clade X, whether by a few thousand years (geologically negligible) or many million. Molecular clocks, of course, attempt to date clade divergence.

Once it is accepted that molecular dates are dates of origin, and that paleontological dates always postdate them, then the arguments about error bars and "acceptable" and "unacceptable" dates become less acute. Paleontologists estimate minimum constraints on the ages of clades (37, 57, 58). Providing the fossil is correctly assigned to a clade, and providing its provenance is known, the date reflects simply current best knowledge on the age of the geological formation that contains the *oldest phylogenetically secure fossil*. We emphasize this point because compendia of fossil dates for the inception and demise of clades (e.g., 59) are littered with records that are optimistically interpreted. The oldest fossil records of a clade will always be phylogenetically uncertain because, by definition, they will exhibit the fewest of all characters to justify membership. This problem is further exacerbated by the fact that the oldest possible records are also very often extremely poorly preserved fragmentary fossils; indeed, they may be little more than a fragment (28). For instance, the oldest records of the shark lineage are a series of isolated scales (60), not the complete articulated skeleton that we might prefer.

In such circumstances, it can be difficult to distinguish between fossils that fail to exhibit diagnostic characters because they are primitive, and fossils that preserve few characters at all for reasons of fossil preservation (*25*). Thus, it is important that molecular clocks are calibrated using phylogenetically secure fossil records, demonstrating the primitive absence of a number of crown-group diagnostic characters, even at the expense of alternative dates, based on poorer data, that are maybe tens of millions of years older.

If fossil dates are accepted as minimum constraints (*3, 4*), their attendant errors must still be considered; but it is the youngest limit of the resulting age span that should be adopted as the minimum age constraint for a lineage split. As such, no fossil date provides a poor minimum constraint—unless it is actually older than the lineage divergence that it purports to date.

Returning to the infamous mammal–bird split, corresponding to the origin of the clade Amniota, the minimum constraint is the minimum age of the Joggins Formation (in which occurs the oldest diapsid and synapsid, respectively, *Paleothyris* and *Protoclepsydrops*). The age span of this geological formation is 314.5–313.4 Ma ± 1.1 myr, a date based on biostratigraphy (palynology) and exact dating from elsewhere, resulting in a minimum constraint of 312.3 Ma (*3*). Again this does not mean that the divergence could not have happened earlier, only that it could not have happened later.

Calculating soft maximum constraints and codifying probability densities

A consequence of accepting that fossils provide only minimum constraints, rather than direct calibrations on molecular clock analyses, is that it is desirable to somehow capture a soft maximum constraint on the calibration of the clock. Various authors have suggested that this can be achieved by codifying an arbitrarily diminishing probability density extending back in time from the minimum constraint (*57, 61–64*). The manner in which these constraints are established and the nature of the variance in probability density can be better informed by paleontological data.

Benton and Donoghue (*3, 4*) have suggested that soft maximum constraints can be established broadly in the manner that Reisz and Müller (*37*) suggested to establish the older error on calibrations. Note, however, that one of the recommendations by these authors was to use the date of the oldest fossil belonging to the nearest relative (sister group) as a guide to the maximum constraint;

as noted elsewhere (*65*), this does not provide a maximum constraint, merely a minimum constraint on that nearest relative's lineage. Probability densities may be estimated using models of diversification and preservation probability below the oldest known fossil in a clade (*66, 67*) and/or informed by older, more tenuous records of the existence of clades, otherwise rejected because they are phylogenetically less secure (Fig. 2). Crucially, it is now possible to implement such constraints without precluding the possibility that the timing of divergence predates the "soft" maximum constraint, but with the assumption that it is increasingly improbable with increasing departure from this constraint (*62*).

Tree making and the molecular clock
Variable rates

In the early years of dating trees, the molecular clock was assumed to be constant (*68, 69*). It soon became clear, however, that the clock ran at different rates both within and between lineages (*70, 71*) and this at first led some to doubt the possibility of using the molecular clock to calibrate dates on a tree. However, estimating divergence times need not depend on a constant clock, and techniques were developed to test for variable rates (*72–74*). These techniques allowed analysts to determine typical rates within the clade of interest, and to eliminate those that deviated from the norm. Unusually fast-evolving genes in particular could give anomalously ancient divergence dates if they were not recognized, and so it was often recommended that such genes should be eliminated (e.g., *75, 76*).

The clock tests, however, lack power for shorter sequences and for genes with low rates of change, and they detect only a small proportion of cases of rate variations in the kinds of genes commonly used for molecular clock studies (*77–79*). For this reason, current tree reconstruction and dating techniques are "relaxed" in that they allow analysts to assume any number of local molecular clocks within a phylogeny (*80*). Some of these techniques assume that evolutionary rates among closely related lineages are similar, or "autocorrelated" (*81, 82*), but this assumption has been rejected by others (e.g., *63*) who recommend methods that allow independent rates as a first pass, and then evaluate whether the reconstructed rates are autocorrelated (*49, 63, 83*).

Further advances in methods of tree reconstruction and dating that allow for the vagaries of evolution and
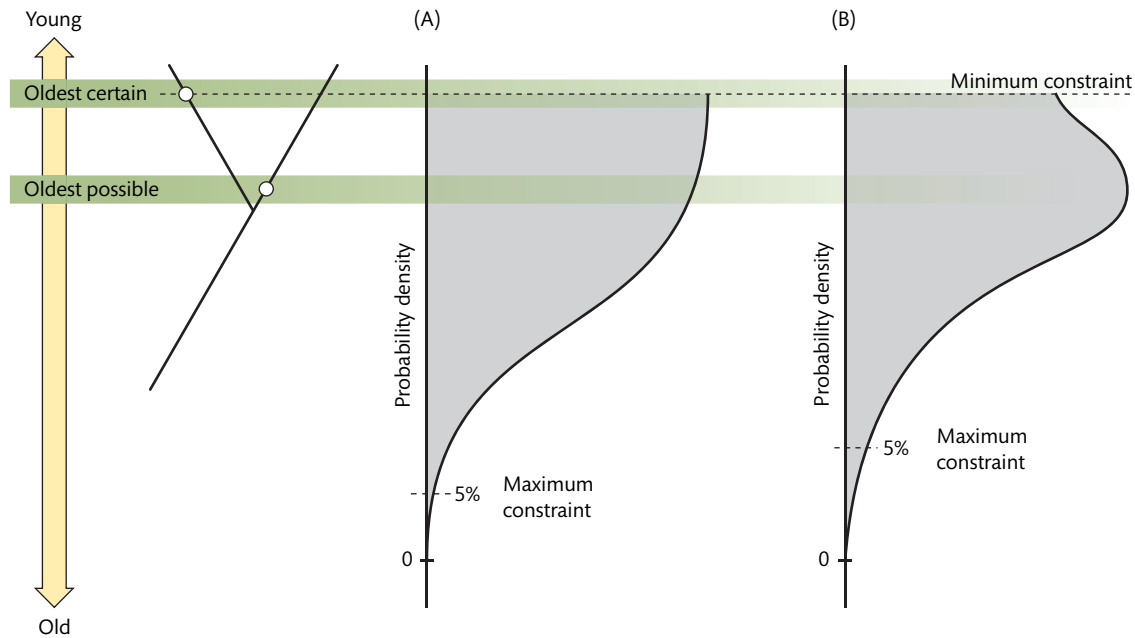
Fig. 2 Two hypothesized patterns for the distribution of probabilities between the soft maximum and minimum constraints on the date of origin of a clade. In (A) the curve is a logistic, corresponding to a standard birth–death model of diversification and an equilibrium at "normal" diversity, when fossils become abundant. In (B) an assumption is added that there might be some older possible fossils, corresponding to an expansion of the probability distribution.

multiple uncertainties include explicit modeling of the evolution of the rate of evolution using Bayesian methods (82–84), the use of nonparametric or semiparametric models of rate evolution (81, 85), and methods that allow for phylogenetic uncertainty as well as rate uncertainty (63, 86). These methods often lead to better concordance overall between molecular and paleontological dates (e.g., 40, 87, 88), but this is not always uniformly the case. For example, Hedges and Kumar (89) argue that the majority of dates in vertebrate evolution agree well between their molecular analyses and the fossils, but certain dates, such as those for the origin of modern mammals and birds, are more ancient than the oldest fossils. On the other hand, Kitazoe *et al.* (49) argue that when abrupt changes in mutation rate and convergent evolution are taken into account in models of molecular evolution over time, divergence estimates for mammals correspond more closely to the fossil record than previously reported. For example, Kitazoe *et al.* (49) estimate the common ancestor of placental mammals at ca. 84 Ma, slightly older than the molecular clock estimate reported by Douzery *et al.* (45), and much younger than other clock estimates of 105 Ma (51) or 129 Ma (50). Concordance between largely independent data sets, such as here, might be a guide to the quality of the result, but need not be of course.

Finding the correct answer

How are we to judge the current state of tree making and tree dating? One view is that the new methods, notably Bayesian approaches and relaxed clocks, must be superior to other techniques because they generate better concordance between paleontological and molecular evidence (e.g., 90, 91), an assumption that the traditional evidence is a yardstick against which new methods and new evidence may be assessed. Of course, in tree making and tree dating there can only be a single answer, a single correct tree, and a single date for any branching point. Others argue that the new methods are so accommodating, one might even claim that they are so relaxed as to be laid back, that they can produce any desired outcome (e.g., 92). A key point about relaxed clock methods is that although there are more inherent assumptions, they are statistically weaker, but in assuming so much about the evolutionary process when the diversity of competing models betrays how little we understand of it, the concern is that our multifarious assumptions will preclude us from better knowing it (93).

Counterintuitively perhaps, relaxation of assumptions need not produce answers that are so broad as to be meaningless. For example, theoretical and empirical studies into the use of wide confidence intervals on multiple calibration dates by Yang and Rannala (*62, 94*) have shown that these have self-correcting properties. They show that hard calibration dates may conflict and produce unsatisfactory results, but dates expressed with at least one soft bound (the soft maximum constraint) interact so as to correct poor calibrations. Poor calibration data expressed with unjustifiable hard confidence intervals may, on the other hand, produce results with misleadingly high precision. These results are encouraging because they show that flexible calibration data, expressed in line with paleontological reality, can interact to cancel out a great deal of the uncertainty. This can then feed back information to the paleontologists that certain calibration dates appear to work better than others, and that the poor dates require closer study to see why they do not work so well. The example of cross validation of calibrations from Douzery *et al.* (*45*) illustrates this point nicely.

The search for the single answer must also apply tree-testing techniques with equal rigor to both morphological/paleontological and molecular trees and dating evidence. There are issues of quality and quantity of data. Statistically speaking, the best data matrix is the largest—more data can lead to better statistical measures. In particular, estimates of divergence times are improved by dense packing of calibration points around the node of interest. But, are more genes and more species always best? Yes: but more progress may be made through the addition of further paleontological constraints, or by further constraining established constraints (*94*).

Quantity and quality

The focus of future work must pursue quantity and quality of data equally. It is self-evident that data sets must increase in size, with the inclusion of ever more species and genes, and larger numbers of calibration dates. But, data quality ought to be determined based on biological and geological criteria, not on statistical expediency. On the whole, taxa should not be deleted from analyses unless there are serious doubts about the accuracy of data, for example from an incomplete or damaged fossil specimen. A statistically "rogue" taxon that has an unusually bad effect on a cladistic analysis (e.g., *95, 96*) is worthy of attention: it must fit in the tree somewhere, and cannot simply be regarded as awkward and

so eliminated. Certain genes, like certain morphological characters, can be shown to contain no phylogenetic signal, and so should be omitted on the basis of such auxiliary evidence. Ornithologists have realized for decades that certain kinds of characters, such as plumage color, are generally not helpful in determining deep phylogenetic relationships, and these are rarely included in morphological phylogenetic analyses. Certain individual genes, or even classes of genes, may similarly be accepted as phylogenetically uninformative at particular levels, and so can only confound a statistical analysis.

We have argued, as have others (e.g., *3, 36–38, 41, 57*) that more calibration dates are generally better than few. But the quality of those dates, or the data they are based on, is critical (*37*). Quality of calibration constraints may be assessed subjectively, based on accuracy of identification of fossils, distinction of crown clades from stems, cross-checking of geological dates, and evaluation of the extent of a well-documented underlying fossil record. We do not believe there is an objective way to determine that one constraint is good, and another is bad; the only bad constraint predates (in the case of a minimum constraint) or postdates (in the case of a soft maximum constraint) the evolutionary event in question. The use of hard minimum constraints and soft maximum constraints, as recommended here, is a more meaningful representation of the nature of paleontological evidence than the attempt to present single fossil-based dates as the holy grail, whether with or without error bars.

At present, tree making and tree dating is often a two-step process, where a single model tree is generated, and dates are then calculated against that tree. Better approaches for the future will probably be to combine the two steps, to allow uncertainty in the tree topology and in the calibrations and calculated dates (*63, 97*). The best solution must maximize the fit of topology and dates against external evidence. Fixing the tree first, and the dates second could well mean that we miss an even better resolution of the data.

The reasons for the two-step process are (a) tractability and (b) distinguishing rates from time. Typically, analysts use one program, or set of programs, to calculate the tree that best fits their data, and then a separate program or programs to apply calibration dates and calculate unknown divergence times. There is no fundamental reason, however, that the two steps could not run in parallel within a single process of calculation, except when the class of data from which rates of evolution are typically calculated (i.e., DNA sequences) are missing entirely for elements of the tree to be built (i.e., fossils).

Distinguishing rates from time is harder. A long branch in a tree could represent an ancient divergence time or a fast-evolving genome. These alternatives can be tested against different genes: if the branch is always long, then an ancient divergence time is the more likely explanation. Fossil dates can also help to distinguish rates from time in some cases. Theoretical studies (*62, 94*) suggest that, with an infinite amount of sequence data, uncertainties in time estimates usually reflect uncertainties in fossil calibrations rather than in branch lengths.

Key calibration dates for molecular clock practitioner

Benton and Donoghue (*3*) presented a set of 30 calibration dates for key genomic animals, from nematode to human, increased to 40 by Donoghue and Benton (*4*).

These have been corrected, revised, and updated in light of new data, and augmented by 25 further calibration dates required because of genome sequencing projects that are approaching maturity, giving a current total of 65 dating arguments (Fig. 3A and B). Further revisions will be provided at http://www.fossilrecord.net.

It is striking that in the short time since the publication of Benton and Donoghue (*3*), revisions have had to be made to some of their paleontological date estimates. These revisions arise more from clarifications of previously incompletely determined materials rather than from outright errors. In particular, two groups of early mammals, the zhelestids and zalambdalestids, had been attributed to many locations in the tree of basal placental mammals; in some analyses they have been interpreted as evidence for an early date of the minimum constraint on several fundamental branching
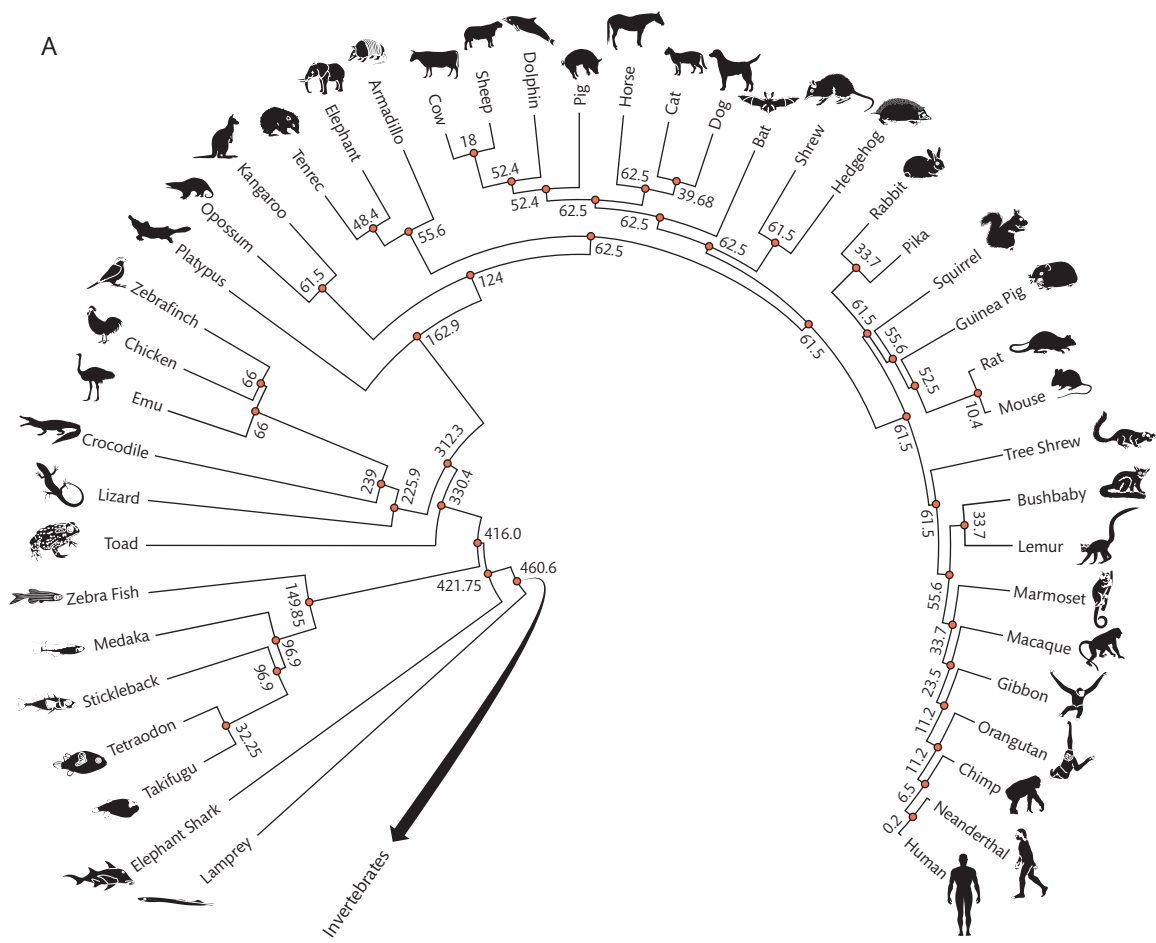

Fig. 3 *Continues*

points within Eutheria. Zhelestids and zalambdalestids are each represented by several species from mid- to late Cretaceous localities in central Asia, with an oldest occurrence around 95.3 Ma. Archibald (*98*) and Archibald *et al.* (*99*) associated these taxa with crown-placental lineages, specifically zhelestids with "ungulatomorphs," a grade that includes hoofed artiodactyls and perissodactyls, and zalambdalestids with Glires (i.e., rodents and lagomorphs). The latter hypothesis dates to the 1960s (*100, 101*). However, some older assessments of Cretaceous mammal affinities (e.g., *102* on zalambdalestids) as well as more recent phylogenetic analyses sampling an adequate number of both recent and fossil clades (e.g., *48, 103–106*) have indicated instead that no Cretaceous taxon has an exclusive, sister taxon relationship to any single crown-placental clade.

The revised interpretation of zhelestid affinities reduces four of the hard minimum age estimates presented in Benton and Donoghue (Table 1 in ref. *3*), namely for "cow–dog," "human–cow," and "human–armadillo," from Late Cretaceous to Paleocene, and for "human–tenrec" from Late Cretaceous to early Eocene. Zalambdalestids had not been used as minimum constraints in Benton and Donoghue (*3, 4*).

Dates for calibrating and constraining molecular clocks

The paleontological constraints provided here were selected because they represent the divergence events between metazoans whose genomes have been sequenced. The reasoning underpinning this is that molecular clock analyses are usually codified on the basis of available data and, thus, these constraints will be of the greatest utility to the greatest number of analysts.

The constraints do not attempt to date the individual fossil on which they are based. Rather, they establish firm minimum and soft maximum constraints on the timing of the component divergence events. Thus, in contrast to common practice, minimum constraints are not established on the precise age, or the maximum possible age of the oldest phylogenetically secure member of the two lineages that result from the divergence event that is to be constrained. Rather, we attempt to derive the minimum possible date of this fossil. If the only stratigraphic constraint available for a fossil is for instance Danian, the minimum constraint would be provided by the geochronological age of the top of the Danian Stage. This is 61.70 Ma ± 0.2 myr (*107*) and, thus, the quoted minimum
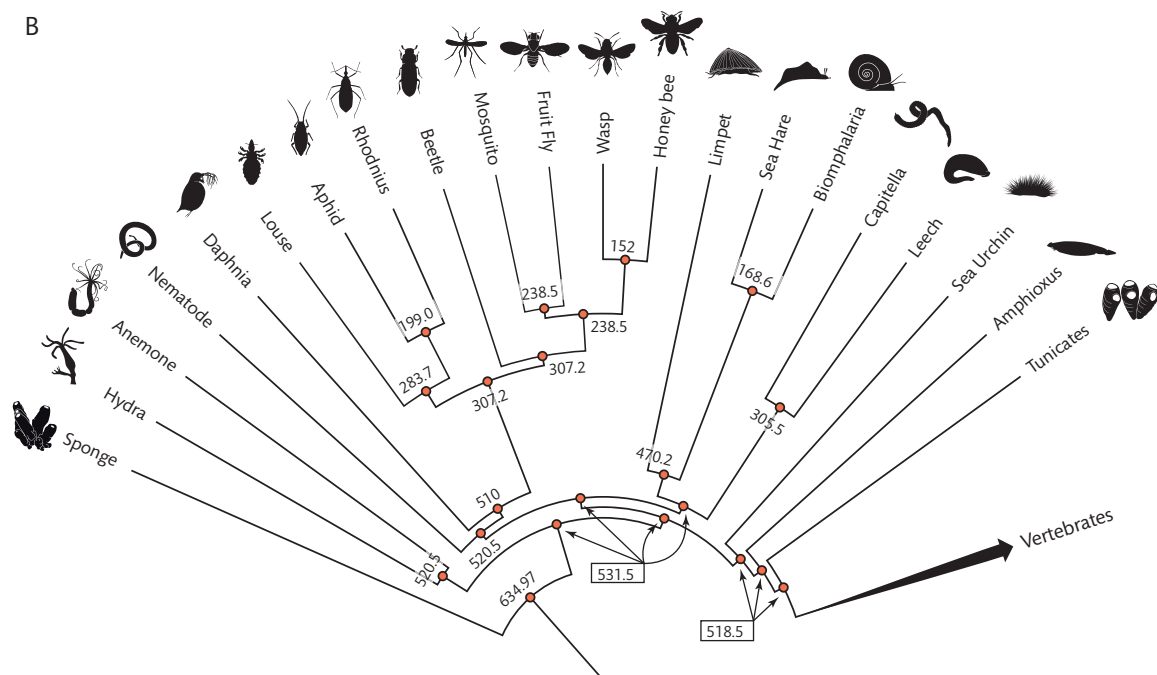

Fig. 3 Summary of paleontological constraints (in myr) on metazoan phylogeny. A. Vertebrates. B. Invertebrates. The dates are minimum constraints, and soft maximum constraints are given in the text, and at http://www.fossilrecord.net/.

constraint is 61.5 Ma. This principle is explained graphically in Fig. 4. Soft maximum constraints are established on the basis of well-preserved assemblages of more plesiomorphic (ancestral) relatives of the clade that lack members of the clade itself. The maximum possible age of that assemblage would be quoted. Thus, if the only stratigraphic constraint on the age of such an assemblage was again Danian, the soft maximum constraint would be would be provided by the geochronological age of the base of the Danian Stage. This is 65.5 Ma ± 0.3 myr (*107*) and, thus, the quoted soft maximum constraint is 65.8 Ma.

It should, but often does not, go without saying that the trees on which paleontological constraints were determined should be compatible with the molecular phylogenies in which they are employed. In the paleontological constraints presented below, most were established within an uncontroversial phylogenetic framework. However, certain nodes have recently undergone revision and so we emphasize here the phylogenetic schemes followed in deriving a constraint in such instances.

The high-level mammalian clades identified herein are based on the topology figured by Springer and Murphy (*108*; Fig. 1); interrelationships of deuterostomes and jawless vertebrates follow Bourlat *et al.* (*109*); other clade relations are not contentious. Terminology for high-level mammalian clades is based on Simpson (*110*), Waddell *et al.* (*111*), and Springer and Murphy (*108*). Note that there is no convention for the conversion of traditional taxonomic concepts, based on the classification of living constituents alone, to crown or total-group-based definitions with the inclusion of fossil taxa, especially stem-taxa. Hennig (*25*) argued that traditional taxa should be converted to total-group definitions but, in practice, different approaches have been adopted, without justification, in different scions in the Tree of Life. For instance, among tetrapods and plants, traditional taxa have generally been converted to crown-based definitions while, elsewhere, total-group definitions have been adopted (*28*). In what follows, we have followed convention and there is no consistency with regard to the adoption of total or crown-based definitions of taxa. However, in each instance we are explicit with regard to the composition of the clades for which we provide constraints on divergence timing.

Homo sapiens: human–neanderthal (minimum = 0.2 Ma; soft maximum = 1 Ma)
H. sapiens neanderthalensis represents an anatomically distinctive European, antedating the European arrival of anatomically modern humans by many thousands of years.

The oldest neanderthal fossils date to just under 0.2 Ma from France (Biache St. Vaast) and Germany (Ehringsdorf) (*112*). The oldest anatomically modern members of our own subspecies are probably remains from the Levant, from the Skhul cave in Israel, dating to ca. 0.100–0.135 Ma (*113*). Because these ages postdate the Geologic Time Scale 2004 (GTS2004) marine timescale (*114*), we tentatively rely on the radiometric and faunal dates provided in the primary literature. Hence, the paleontologically minimum date for this split may be estimated based on the older, undisputed neanderthal sites in Europe at 0.2 Ma.

For the soft maximum split within *H. sapiens*, we would suggest the widespread occurrences of *H. erectus* outside Africa over 1 Ma (*112*). In the case of central Asia, there appear to have been populations of *H. erectus* nearly 1.8 Ma (*115*). Despite occurrences of the genus *Homo* throughout Asia and Africa by around 1 Ma, no evidence for neanderthals or anatomically modern humans from this time is yet known (*112*).

*Hominoidea: chimp–human, neanderthal (minimum = 5.7 Ma; soft maximum = 10 Ma)*
The dating of the chimp–human split has been discussed for nearly a century. Early paleontological estimates, up to the 1970s, placed the branching point deep in the Miocene, at perhaps 20–15 Ma, but this was revised dramatically upward to about 5 Ma by early molecular studies (*116*), and estimates as low as 2.7 Ma have been quoted (*117*). Paleontological evidence for the branching point was distinctly one-sided until recently, since the only fossils fell on the human line, and so the question of the date of divergence of humans and chimps became synonymous, for paleontologists, with the date of the oldest certain hominin (species on the human, not chimp, line). The oldest chimpanzee fossils are, at ca. 0.5 Ma, comparatively young (*118*).

The date of the oldest hominin has extended backward rapidly in the last 25 years. Until 1980, the oldest fossils were gracile and robust australopithecines from 3 Ma. The discovery of "Lucy," now termed *Praeanthropus afarensis* in Ethiopia (*119*) extended the age back to 3.2 Ma at most. Then, two further hominin species pushed the age back to over 4 myr: *Ardipithecus ramidus* from rocks dated as 4.4 Ma from Ethiopia (*120*) and *Praeanthropus anamensis* from rocks dated as 4.1–3.9 Ma from Kenya (*121*). More recent finds, remarkably, have pushed the dates back to 6 myr: *A. ramidus kadabba* from Ethiopia (5.8–5.2 Ma; *122*), *Ororrin tugenenis* from Kenya (c. 6 Ma; *123*), and *Sahelanthropus tchadensis* from Chad (6–7 Ma; *124*). The last two taxa have proved highly controversial, with claims that one or other, or both, are not
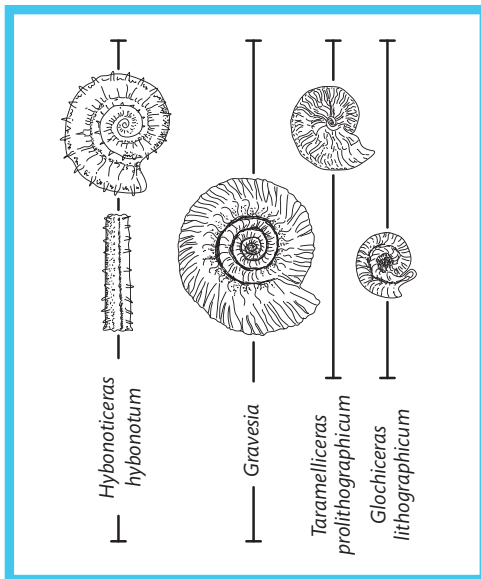
1. Oldest certain fossil in lineage
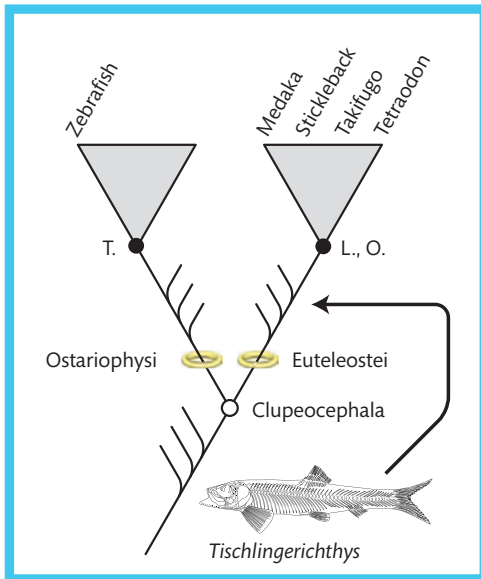
2. Lithostratigraphy of formation
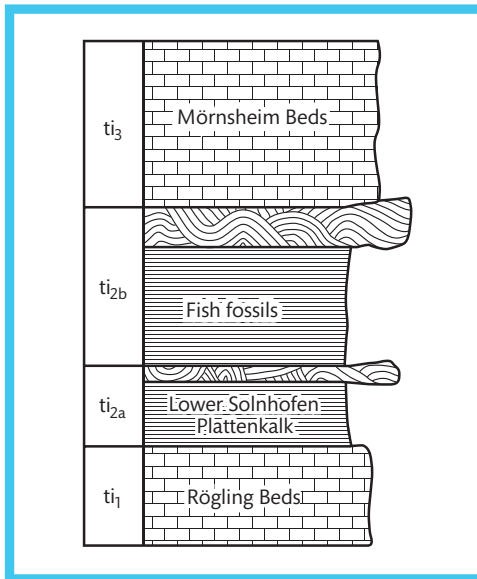
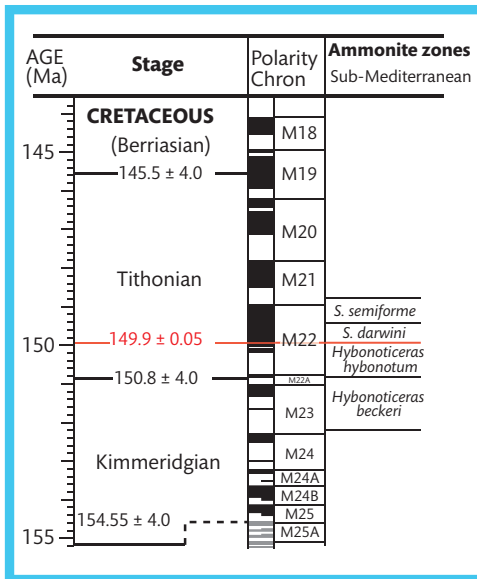3. Biostratigraphy

4. Chronostratigraphy

Fig. 4 Graphical summary of the process by which a time constraint on lineage divergence is derived, using the split between zebrafish and euteleost models as an example. 1. The oldest phylogenetically secure fossil is identified that falls within the overall clade circumscribed by the lineage divergence. In this instance it is *Tischlingerichthys*, among others; there is equivocation over its membership of any component of the crown euteleost lineage, but it is clearly a member of the euteleost total-group. 2. The stratigraphic age of the fossil taxon is established: *Tischlingerichthys* has been recovered from zone ti_{2b} of the Solnholfen Limestones. 3. A means of correlating the stratigraphic age of the fossil is established: the strata bearing *Tischlingerichthys* also bear the ammonite *Hybonoticeras hybonotum*, which is a diagnostic zone fossil for the lower Tithonian. A minimum age for *Tischlingerichthys* can be obtained from the base of the succeeding *S. darwini* ammonite biozone since the Solnholfen Limestones fall fully within the *H. hybonotum* Biozone. 4. The minimum age for the oldest record of *Tischlingerichthys* is established using the 2004 Geologic Timescale in which the base of the *S. darwini* Biozone has been determined to correlate to the base of the M22n magnetostratigraphic polarity chron, which has itself been dated at 149.9 Ma ± 0.05 myr. Thus, the minimum constraint on the divergence of Ostariophysi and Euteleostei is the minimum age interpretation of the errors on this date, equating to 149.85 Ma. As tortuous as this argumentation appears, the majority of splits require many more steps of lithostratigraphic, biostratigraphic, chemostratigraphic, and/or magnetostratigraphic correlation before they can be tied to a geochronological age (*4*).

hominin, but ape-like (*125*). Recently, evidence has been presented to bolster the case that *Orrorin*, at least, is in fact a hominin (*126*).

Dating of the *Sahelanthropus* beds in Chad is not direct. Biostratigraphic evidence from mammals in particular, but with cross-checking from fish and reptile specimens, indicates that the unit is definitely late Miocene (i.e., older than 5.33 Ma), and that it may be older than the Lukeino Formation of Kenya, the source of *Orrorin* (dated at 6.56–5.73 Ma from Ar/Ar dates on volcanic layers; *127*), equivalent to the lower fossiliferous units of the Nawata Formation at Lothagam (dated as 7.4–6.5 Ma; *128*). This would suggest a date for the sediments containing *Sahelanthropus* of 7.5–6.5 Ma, based on biostratigraphy and external dating. However, since the status of *Sahelanthropus* remains contentious, we based our minimum constraint on *Orrorin,* which has minimally an age of 5.73 Ma (*129*). Thus, we determine a 5.7 Ma age for the minimum constraint on the human–chimp split. Kumar *et al.* (*130*) have recently calculated a range of ages for the human–chimp divergence of 4.98–7.02 Ma; their minimum constraint (4.98 Ma) is younger than the oldest fossils (*Orrorin*, *Sahelanthropus*).

The report by Suwa *et al.* (*131*) on a late Miocene fossil gorilla hints at a similar age for the gorilla and orangutan lineages. Some late Miocene ape fossils, such as *Gigantopithecus* and *Sivapithecus* may be stem-orangs. Nonetheless, a range of such apes, *Ankarapithecus* from Turkey (10 Ma), *Gigantopithecus* from China (8–0.3 Ma), *Lufengopithecus* from China (10 Ma), *Ouranopithecus* from Greece (10–9 Ma), and *Sivapithecus* from Pakistan (10–7 Ma) give maximum ages of 10 Ma, early in the late Miocene, and these deposits have yielded no fossils attributable to either chimps or humans. This is taken as the soft maximum constraint on the human–chimp divergence.

Pongidae: orangutan–chimp, human, neanderthal (minimum = 11.2 Ma; soft maximum = 33.7 Ma)
While there are numerous taxa of fossil hominoids from Africa and Eurasia, few can be unambiguously attributed to an extant ape lineage; and most of these are hominids. Numerous fossils attributable to *Pongo* are known from Pleistocene sites in east Asia; and another fossil orangutan relative, *Gigantopithecus*, is known from the late Miocene and Pliocene (*132*). *Sivapithecus* from the Miocene of southern Asia is the oldest ape fossil interpreted with some confidence as a close relative to orangutans (*132*). There is a possible, but tentative, record of a fossil orangutan relative dating to the late Oligocene in

northern Kenya (*133*). The oldest definitive occurrence of an orangutan relative is *Sivapithecus* from the Chinji Formation of Pakistan, corresponding to magnetic polarity chron 5Ar, estimated to be ca. 12.5 Ma before present (*134*). This correlates to the Serravallian stage, the top of which is at 11.2 Ma, our minimum estimate for the divergence of the orangutan from other great apes.

As a soft maximum we suggest the first diverse occurrence of anthropoids from the earliest Oligocene of the Fayûm, Egypt. These primates comprise a diverse radiation just on the Oligocene side of the Eocene–Oligocene boundary at 33.7 Ma (*135*), and lack derived features of the extant great ape lineages.

Hylobatidae: gibbon–orangutan, chimp, neanderthal, human (minimum = 11.2 Ma; soft maximum = 33.7 Ma)
Numerous taxa of Miocene apes may share a close relation with extant gibbons to the exclusion of other hominoids. European taxa such as *Dryopithecus* and *Oreopithecus*, with a record dating to the middle Miocene, have over the years occasionally been linked to hylobatids (*132*). However, recent cladistic analyses do not place any of these taxa with hylobatids to the exclusion of other catarrhines (*136*). Hence, gibbons cannot be said to have a definitive fossil record before Pleistocene occurrences of the extant genus in east Asia; and previously named Miocene species of *Pongo* are now recognized under other Miocene hominoid genera (*137*). The cladistic divergence of hylobatids from other catarrhines is therefore constrained by the record of great apes from South Asia (see *Sivapithecus* discussed earlier), minimally dated at the top of the Serravallian at 11.2 Ma. Divergences within hylobatids themselves would of course be much younger.

As a soft maximum we would again suggest the first diverse occurrence of anthropoids from the earliest Oligocene of the Fayûm, Egypt. These primates comprise a diverse radiation just on the Oligocene side of the Eocene–Oligocene boundary at 33.7 Ma (*135*), and lack derived features of extant hominoids.

Catarrhini: macaque–gibbon, orangutan, chimp, neanderthal, human (minimum = 23.5 Ma; soft maximum = 34.0 Ma)
The human–macaque split is equivalent to the branching of Old World monkeys (Cercopithecoidea) and apes (Hominoidea), which together form the clade Catarrhini.

The oldest cercopithecoids are *Victoriapithecus macinnesi* from Kenya, and two species of *Prohylobates* from Libya and Egypt. Miller (*138*) surveyed all fossils of these

two genera, and compared ages of their respective deposits. The oldest cercopithecoid fossil is a tooth identified as *Victoriapithecus* sp. from Napak V, Uganda (c. 19 Ma), followed by *Prohylobates tandyi* from Moghara, Egypt (18–17 Ma) and *Prohylobates* sp. from Buluk, Kenya (>17.2 Ma), *Prohylobates simonsi* from Gebel Zelten, Libya (c. 17–15 Ma), and *V. macinnesi* from Maboko, Kenya (ca. 16–14.7 Ma). MacLatchy *et al.* (*139*) report an even older cercopithecoid, a fragment of a maxilla from the Moroto II locality in Uganda, which has been radiometrically dated to be older than 20.6 Ma ± 0.05 myr (*140*).

The oldest hominoids include *Morotopithecus*, also from the Moroto II locality in Uganda (*140*). Young and MacLatchy (*141*) determined that this taxon is a hominoid, located in the cladogram above the gibbons, and so not the most basal member of the group. Because of incompleteness of the material, Finarelli and Clyde (*142*) are less certain of its phylogenetic position, but *Morotopithecus* is certainly a catarrhine. Even older is the first record of the long-ranging hominoid genus *Proconsul* from Meswa Bridge in Kenya, biostratigraphically constrained to ~23.5 Ma (*143, 144*). Even older still is the purported hominoid *Kamoyapithecus* from the Eragaliet Beds of the Lothidok Formation of Kenya, dated at 24.3–27.5 Ma (*145*), but the material is insufficient to determine whether it is a hominoid or a catarrhine, possibly lying below the human–macaque split (*142*).

So, the minimum constraint on the human–macaque split is 23.5 Ma, based on the oldest record of *Proconsul*, biostratigraphy and external dating. The soft maximum constraint is based on members of the stem of Catarrhini, namely the Families Propliopithecidae (*Propliopithecus*, *Aegyptopithecus*) and Oligopithecidae (*Oligopithecus*, *Catopithecus*) that are basal to the cercopithecoid–hominoid split (*146*). These are represented in particular from the rich Fayûm beds in Egypt, which possess a diverse anthropoid primate fauna, including stem platyrrhines and catarrhines (*147*) during the early Oligocene ((*135*); 33.9–28.4 Ma ± 0.1 myr). Hence, at the base of the Oligocene at 33.9 Ma ± 0.1 myr, the Fayûm shows a diversity of primates and other mammals, but no members of crown-group hominoids or cercopithecoids.

Anthropoidea: marmoset–macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 33.7 Ma; soft maximum = 65.8 Ma)
The oldest South American primate is *Branisella* from the late Oligocene of Bolivia; this taxon cannot be more explicitly linked to any of the modern platyrrhine groups

(*132*). The middle Miocene locality of La Venta, Columbia, has produced the oldest remains of essentially modern platyrrhines, including the possible marmosets *Micodon*, *Patasola*, and *Lagonimico* (*148*). The oldest crown anthropoid, that is, the oldest primate reconstructed within the clade of living New and Old World monkeys, consists of the Fayûm taxon *Catopithecus* from the Fayûm Quarry L-41, now dated at the end of the Priabonian (*135*) with an upper bound of 33.7 Ma. Older taxa such as African *Altiatlasius* and *Biretia*, and Asian eosimiids and amphipithecids, may in fact be anthropoids, but appear to fall outside crown Anthropoidea (*147*).

Given the fact that the oldest known euprimate (*Altiatlasius*) has been regarded tentatively as an anthropoid sister taxon (*147*), the soft maximum for anthropoids must predate this occurrence in the late Paleocene. Early Paleocene strata have yielded fossils of several groups (plesiadapids, paromomyids, carpolestids) reconstructed closer to crown primates than to Scandentia or Dermoptera, but have not yielded any definitive crown primates. Hence, the paleontological soft maximum can be defined by the base of the Paleocene, at 65.8 Ma.

Primates: bushbaby, lemur–marmoset, macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 55.6 Ma; soft maximum = 65.8 Ma)
Crown-group Primates, or Euprimates, encompass living forms plus the extinct adapoids and omomyoids, as the latter are more closely related to extant lemuriforms than to anthropoids (*149, 150*). The oldest fossil often attributed to primates is *Purgatorius* known from the basal Paleocene (64 Ma ± 1 Ma), reputed from time to time to have been latest Cretaceous in age. However, the latest Cretaceous specimen is an unidentifiable tooth (*150*). The oldest euprimate is *Altiatlasius* from the late Paleocene of Morocco (*151*), and is recognized as the most basal stem anthropoid (*147, 152*) or, alternatively, as the most basal euprimate (*150*). Broadly speaking, this specimen is currently regarded as the oldest euprimate fossil. *Altiatlasius* comes from the Adrar Mgorn 1 locality in the Ouarzazate Basin of Morocco, dated generally as late Paleocene (Thanetian stage). Magnetostratigraphic study (*153*) narrows the age range of the locality to "late or latest Thanetian," and so the age of the top of the Thanetian Stage provides the minimum constraint. This is 55.80 Ma ± 0.2 myr (*107*), and so the minimum constraint is 55.6 Ma.

The soft maximum constraint may be marked by older possible primate fossils. McKenna and Bell (*154*) implied in their classification that carpolestids, with a record in

the Danian (early Paleocene) are euprimates, but this has not been substantiated elsewhere. They also attribute the basal Paleocene *Decoredon* from China to Primates, although this has been regarded as a hyopsodontid by others (e.g., *155*). In general, early Paleocene strata have yielded fossils of several groups (plesiadapids, paromomyids, carpolestids) reconstructed closer to crown primates than to Scandentia or Dermoptera (*150*), but have not yielded any definitive crown primates. Hence, the paleontological soft maximum constraint can be defined by the base of the Paleocene, at 65.8 Ma.

Strepsirhini: mouse lemur–bushbaby (minimum = 33.7 Ma; soft maximum = 55.6 Ma)
Malagasy primates are extraordinarily diverse, but nevertheless comprise an extant radiation that shares a single common ancestor to the exclusion of other primates such as galagos, lorises, and monkeys. There is an extraordinary diversity of subfossil primates from Madagascar, but neither these nor other lemuriforms have a fossil record demonstrably older than the Holocene (*152*). In contrast, the lorisiform sister-radiation of Malagasy lemurids (including galagos and bushbabies) shows a less ambiguous fossil record through the late Eocene, including the oldest known records of the toothcombed prosimians from the Birket Quarun Formation of the Fayûm, Egypt [e.g., *Karanisia*, Seiffert *et al.* (*156*)]. Following Seiffert (*135, 152*) this unit corresponds to the Priabonian with an upper bound of 33.7 Myr before present.

Fossil primates are relatively common mammalian fossils at many localities in North America and Eurasia throughout the Eocene; yet toothcombed prosimians remain conspicuously absent before the end of the middle Eocene. Hence, we suggest the first appearance of euprimates, represented by *Altiatlasius* from the late Paleocene of Morocco at 55.80 Myr ± 0.2 myr (*102*) (or 55.6 Myr) as the soft maximum for Strepsirhini.

Archonta: tree shrew–bushbaby, lemur, marmoset, macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 61.5 Ma; soft maximum = 131.5 Ma)
Tree shrews are members of Scandentia, an order within Archonta (= Euarchonta) that has long been seen as a close relative of Primates. Current trees (e.g., *51, 150*) place Scandentia as sister group of Dermoptera, and those two as sister group of Primates. The minimum constraint on dating the split between tree shrews and any of the primates is set then by determination of the oldest member of Orders Scandentia, Dermoptera, or Primates or their respective stem relatives.

Scandentia (tree shrews) and Dermoptera (flying lemurs) are small, non-speciose orders of mammals, with sparse fossil records. The oldest scandentian is *Eodendrogale* from the middle Miocene of China (*149*), and the oldest dermopteran is a single specimen from the late Eocene of Thailand, *Dermotherium* (*149*). The oldest Primates are more diverse, and clearly extend the date of origin of the order to the beginning of the Paleocene, as already discussed. Hence, the minimum constraint on the Scandentia–Primates split (i.e., origin of Archonta) is set in the early Paleocene based on Torrejonian occurrences of extinct primate sister taxa such as carpolestids and plesiadapids (*150*). The upper bound of the Torrejonian North American Land Mammal Age (NALMA) correlates to the top of the Danian, 61.5 Ma.

The soft maximum constraint could correspond to the date of origin of Archontoglires, the larger clade including Archonta (primates + flying lemurs + tree shrews) and Glires (rodents + rabbits). However, the problem is that no definitive records of Archontoglires, or any other crown-group placental mammal, exist before the K-T boundary. A tenuous link between ca. 95 Ma zalambdalestids and lagomorphs and/or rodents has been suggested on several occasions (e.g., *99, 101, 157*); but this link has been disputed (e.g., *158*) and is not supported by well-sampled phylogenetic studies (*48, 104–106*). As there is a relatively good fossil record that documents numerous mammalian groups during the Cretaceous, including the stem-Metatheria and Eutheria, but lacking any crown placental, using the criteria outlined earlier we are left with a similar value for both the paleontological minimum and soft maximum in the early Paleocene. Because we do not want to rule out *a priori* the possibility suggested by some molecular clock analyses of an older radiation of crown placental groups deep in the Cretaceous, we have relaxed the criteria for identifying a paleontological soft maximum for supraordinal placental mammal clades. Hence, the next, best-documented therian node lower than Archontoglires that is paleontologically well documented is the divergence between Metatheria and Eutheria, in the Early Cretaceous, represented by the Liaoning fossils of *Eomaia* (*159*) and *Sinodelphys* (*160*). Liaoning fossils of the Jehol biota have been estimated to be between Barremian and Aptian. Importantly, the association of Jehol fossils and dated horizons is not without ambiguity (*161*); hence we conservatively use the age of the Barremian to define the ages of Jehol specimens (*162*), which indicates a lower bound of 130 Ma ± 1.5 myr, or 131.5 Ma.

Archontoglires: rabbit, pika, squirrel, guinea pig, mouse, rat–tree shrew, bushbaby, lemur, marmoset, macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 61.5 Ma; soft maximum = 131.5 Ma)

The human–mouse split is synonymous with the latest branching point between the mammalian Orders Primates and Rodentia. Both orders are members of the clade Archontoglires, sometimes called Euarchontoglires. Archontoglires is composed of two clades, the Archonta and the Glires, and Primates belongs to the former, Rodentia to the latter. Thus, the human–mouse split becomes synonymous with the origin of Archontoglires.

As stated earlier, fossil evidence for this branching point does not exceed 65.2 Ma, the beginning of the Paleogene (base of Cenozoic, base of Tertiary), and corresponding to the extinction of the dinosaurs and the beginning of the radiation of placental mammal orders. Several molecular analyses have suggested that crown-placental orders might have their origin at some point much deeper in the Cretaceous, ranging from over 125 Ma (*74, 163*) to much younger dates that are in line with fossil evidence (*45, 49*).

As stated earlier, the oldest confirmed primates are from the Paleocene–Eocene transition, 55.5 Ma (*164*). The oldest "plesiadapiform"-grade mammals include early Paleocene *Palaechthon* and carpolestids. The oldest undisputed fossil rodents are known with confidence from the Thanetian (late Paleocene, 58.7–55.8 Ma), including members of the Family Ischyromidae from North America and Europe (*101*). Some or all eurymyloids from Asia may fall on the stem to Rodentia and/or Lagomorpha (*48, 165*), which would provide a minimum record for Glires in the early Paleocene, corresponding to the record of *Heomys* from the Shanghuan Asian Land Mammal Age (*166*). The upper bound for the Shanghuan ALMA, as for the Torrejonian NALMA, is the top of the Danian, 61.5 Ma. As stated earlier for the common ancestor of Scandentia, Dermoptera, and Primates, given the lack of crown placental fossils during the Cretaceous, we suggest a soft maximum paleontological estimate for Archontoglires at 131.5 Ma, based on the record of *Eomaia* and *Sinodelphys* from China (see Archonta).

Glires: pika, rabbit–squirrel, guinea pig, mouse, rat (minimum = 61.5 Ma; soft maximum = 131.5 Ma)

The rabbit–mouse split is synonymous with the clade Glires, comprising Orders Rodentia plus Lagomorpha). The date would have been assumed traditionally to lie at 65 Ma, or younger, marking the time of purported placental mammal radiation after the extinction of the dinosaurs (see earlier under Archontoglires).

The oldest crown lagomorphs are somewhat younger. Stucky and McKenna (*101*) indicate several Eocene rabbits from the Lutetian: *Lushilagus* from China, *Procoprolagus* from Canada, and *Mytonolagus* from the United States. Meng and Wyss (*167*) note an older possible stem lagomorph, *Mimotona,* from the early to late Paleocene (Doumu Formation, Nonshangian, Qianshan Basin, China), the same unit that yielded the putative earliest stem rodent *Heomys*.

The minimum constraint on the age of clade Glires, and so for the rabbit–mouse split, based on *Heomys*, is the same as that for Archontoglires, described earlier at 61.5 Ma. Again we suggest a paleontological soft maximum of 131.5 Ma.

Lagomorpha: pika–rabbit (minimum = 48.6 Ma; soft maximum = 65.8 Ma)

The modern order Lagomorpha consists of two groups: leporids (rabbits and hares) and ochotonids (pikas). There are just under a dozen species of pikas (Ochotonidae, *Ochotona*), the oldest relative of which (to the exclusion of leporids) has been reported to be the late Eocene Asian form *Desmatolagus* (*154*). A yet older taxon, *Decipomys* from the early Eocene of central Asia, shows a pattern of enamel microstructure that could be a "structural predecessor" to that of modern ochotonids (*168*). The status of *Decipomys* as an ochotonid, or of Eocene palaeolagids as close relatives of rabbits and hares, would indicate a divergence within crown Lagomorpha by the early or middle Eocene, respectively. A recent analysis of isolated hindlimb elements from China and India (*169*) also indicates that leporids and ochotonids were distinct by the early Eocene. Although fragmentary, these elements are surprisingly diagnostic for the Leporidae. These identifications are consistent with the interpretation of the early Eocene *Strenulagus* and *Gobiolagus* from central Asia as leporids (*170*), although Lopatin and Averianov (*171*) have more cautiously assigned them to "Lagomorpha, Family Strengulidae" without specifying a crown affiliation. Hence, our minimum estimate for crown Lagomorpha is based on the Indian leporid fossils described by Rose and colleagues (*169*) from the middle Ypresian-equivalent Cambay Shale in West-Central India. The top of the Ypresian is dated at 48.6 Ma.

There are many fossil Glires on the stem to Lagomorpha that long predate the first unambiguous occurrence of a leporid or ochotonid (*48, 172*). For the soft maximum divergence of crown lagomorphs we choose the K-T

boundary at 65.8 Ma, based on the occurrence of basal Glires such as *Mimotona* and *Heomys*, reconstructed near the base of modern Rodentia and Lagomorpha. None of these Paleocene Glires can be defended as a member of Leporidae or Ochotonidae.

Rodentia: squirrel–guinea pig, mouse, rat (minimum = 55.6 Ma; soft maximum = 65.8 Ma)
Recent phylogenies place squirrels (sciuromorphs) external to guinea pigs and murid rodents (i.e., Caviomorpha + Myomorpha) (*48, 173, 174*). Ischyromyids dating to the late Paleocene from North America and Europe have been linked to modern sciurids (*154*), which would give a minimum constraint for crown Rodentia of 55.6 Ma. Recent phylogenies do not consistently place ischyromyids such as *Paramys* and extant sciurids in the same clade, but they generally do fall within crown-group Rodentia (*48, 103*).

Early Paleocene eurymylids may not be rodents proper, but members of a larger clade including Simplicidentata, or they may fall outside Simplicidentata, but within Glires, as outgroup to rodents and rabbits (*48*). Therefore, a reasonable soft maximum constraint on the base of crown Rodentia could be set by these early rodents at the base of the Paleocene at 65.8 Ma.

Rodentia (minus sciurids): guinea pig–mouse, rat (minimum = 52.5 Ma; soft maximum = 58.9 Ma)
The guinea pig (*Cavia*), a member of Caviomorpha and Ctenohystrica (*173, 175*), is the closest relative to the Muridae, the family containing mouse (*Mus*) and rat (*Rattus*). Caviomorpha is a member of the larger clade Hystricognatha, and Muridae is a part of the larger clade Myomorpha, which in turn falls in the major clade Sciurognatha, according to traditional classifications. If so, this puts the guinea pig–mouse divergence as equivalent to the origin of crown-group Rodentia. A newer molecular phylogeny makes Myomorpha closest to Hystricognatha, and Sciuromorpha closest to those two (*173*).

The oldest member of the Caviomorpha stem group is *Tsaganomys* from the mid-Oligocene Hsanda Gol Formation of Mongolia (*154, 167*), while the oldest members of the Myomorpha stem group are early Eocene dipodids such as *Ulkenulastomys*, *Blentosomys*, and *Aksyiromys* from the Obayla Svita of the Zaysan Basin, Kazakhstan (*176*). Lucas (*177*) assigns an Irdinmanhan age to this site, based on comparisons of the contained mammals, moving it from early Eocene to the base of the middle Eocene, and with a soft maximum age of K/Ar age of 55–56 Ma (= 52.5–54.7 Ma when corrected to new

IUGS constants) measured from extrusive volcanic rocks that underlie the Toruaygyr mammalian Assemblage, also Idinmanhan in age, in Kirgyzstan. We take the upper end of this range, 52.5 Ma, as our minimum constraint on the guinea pig–mouse split date.

The soft maximum constraint might be taken as equivalent to the age of the ischyromyids and other entirely extinct rodent groups from the late Paleocene (Thanetian) of North America and Europe (*154*), 58.9 Ma.

Muridae: mouse-rat (minimum = 10.4 Ma; soft maximum = 14.0 Ma)
The mouse (*Mus musculus*) and rat (*Rattus norvegicus*) are both members of the Subfamily Murinae within the Family Muridae, members of the larger clade of muroid rodents. The Old World rats and mice are hugely diverse, with over 500 species, and they appear to have radiated relatively rapidly in Europe, Africa, Asia, and Australia.

The phylogeny of all genera within Murinae has not been determined, so the location of the split between *Mus* and *Rattus* is somewhat speculative at present. However, all current morphological and molecular phylogenies (*178–181*), indicate that *Mus* and *Rattus* diverged early in the evolution of Murinae, but not at the base of the divergence of that clade. A lower limit to the mouse–rat divergence is indicated by the oldest known murine fossil, *Antemus chinjiensis* from the middle Miocene Chinji Formation of Pakistan, dated at about 14.0–12.7 Ma on the basis of magnetostratigraphy and radiometric dating (*182*).

The oldest fossil example of *Mus* dates from 7.3 Ma, a specimen of *Mus* sp. from locality Y457 in the Siwaliks (*182*). Fossils of *Rattus* are not known until the latest Pliocene and the Pleistocene of Thailand (*183*) and China (*184*), no more than 3 Ma. The divergence of the two lineages leading to *Mus* and *Rattus* was stated to be 14–8 Ma by Jacobs and Pilbeam (*185*), in a first review of the fossil evidence. This range was narrowed down at its older end to 12 Ma in subsequent studies (*186, 187*), based on the first appearance of the fossil genus *Progonomys*, early members of which were assumed to include the common ancestor of *Mus* and *Rattus*. The 12 Ma figure has most commonly been selected as the mouse–rat calibration point, but dates in the range from 16–8.8 Ma have been used in recent molecular studies.

In a thorough review of the fossil evidence, Jacobs and Flynn (*182*) show that records of *Progonomys* in the Siwalik succession extend from 12.3 to 8.1 Ma, with the later forms (10.4–8.1 Ma) assumed to lie on the *Mus* lineage. The extinct genus *Karnimata* (11.1–6.4 Ma) is interpreted as a member of the lineage leading to *Rattus*. The

oldest record (11.1 Ma) is uncertain, but the next (at 10.4 Ma) is unquestionable. The early species, *Progonomys hussaini* (11.5–11.1 Ma) is interpreted as an undifferentiated basal murine antedating the common ancestor of *Mus* and *Rattus* by Jacobs and Flynn (*182*), and so they place the *Progonomys–Karnimata* split (equivalent to the *Mus–Rattus* split) at not much beyond 11 Ma, "although it may be younger." The dating is based on detailed field stratigraphic study of the long Siwaliks sedimentary sequence, with dating from magnetostratigraphy and radiometric dating (*188, 189*). The soft maximum constraint on this date is taken as the oldest record of *Antemurus* at 14.0 Ma.

Boreoeutheria: hedgehog, European shrew, bat, cow, sheep, dolphin, pig, horse, dog, cat-rabbit, pika, squirrel, guinea pig, mouse, rat, tree shrew, bushbaby, lemur, marmoset, macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 61.5 Ma; soft maximum = 131.5 Ma)

The human–cow divergence is synonymous with the origin of Boreoeutheria. This clade is composed of the clades Archontoglires (human) and Laurasiatheria (cow). A number of Late Cretaceous putative laurasiatherians have been cited. The oldest supposed laurasiatherians have been said to be the zhelestids, from the Bissekty Formation of Dzharakuduk, Kyzylkum Desert, Uzbekistan, and the even older Khodzhakul Formation at Sheikhdzhili, which would provide a very ancient minimum age constraint on the clade (early Cenomanian, 95.3 Ma) if the assignment is correct. Some authors (*99, 190, 191*) place the zhelestids in Laurasiatheria, basal to the hoofed artiodactyls and perissodactyls. This has been challenged, however, and more comprehensive cladistic analyses of basal Eutheria (*104, 105*) place zhelestids outside of the crown clade of extant orders.

As for the other superordinal placental clades indicated earlier, the minimum paleontological constraint for Boreoeutheria is again the early Paleocene, constrained here by the To1 record of *Protictis* (61.5 Ma; see later). The paleontological soft maximum cannot be better constrained than the Early Cretaceous records of *Eomaia* and *Sinodelphys* from the Liaoning beds of China (131.5 Ma; see Archonta).

Laurasiatheria: shrew, hedgehog–cow, sheep, dolphin, pig, horse, cat, dog, bat (minimum = 62.5 Ma; soft maximum = 131.5 Ma)

The split between the lipotyphlans (shrew, hedgehog, mole, and *Solenodon*), on the one hand, and the cow, sheep, whale, pig, horse, cat, dog, pangolin, and bat, on the other, is equivalent to the base of the clade Laurasiatheria.

Arguably, the crown-placental order most commonly regarded among paleontologists to have representatives in the Cretaceous is Lipotyphla. McKenna and Bell (*154*) reported the oldest lipotyphlan as *Otlestes* from the Cenomanian (99.6–93.5 Ma) of Uzbekistan, but Archibald (*190*) regarded it as a basal eutherian, lacking derived characters of Lipotyphla, or any other modern order. More recently, Averianov and Archibald (*191*) synonymized it with *Bobolestes* (from the same local fauna) and regarded it as a questionable zalambdalestoid. Next in time is *Paranyctoides* from the Turonian (93.4–89.3 Ma) of Asia and the Campanian (83.5–70.6 Ma) of North America, and *Batodon* from the Maastrichtian (70.6–65.5 Ma) of North America, both regarded as lipotyphlans by McKenna and Bell (*154*). Archibald (*190*) is uncertain, but retains these records pending discovery of further specimens.

The oldest, relatively uncontroversial records of Laurasiatheria are Paleocene carnivorans (cf. *Protictis*). As above for Carnivora, we tentatively assign an early Paleocene minimum age constraint to this node, corresponding to To1 at 62.5 Ma. The soft maximum constraint is, as for the other supraordinal divergences, 131.5 Ma.

Lipotyphla: European shrew–hedgehog (minimum = 61.5 Ma; soft maximum = 131.5 Ma)

The shrew and hedgehog are members of clade Lipotyphla/Insectivora. They represent, respectively, the larger clades Soricomorpha and Erinaceomorpha. The oldest erinaceomorphs include *Adunator* from the early and late Paleocene of North America (*154*). The fossil record of the other extant lipotyphlans (or "eulipotyphlans"), Talpidae and Solenodontidae, is much younger.

McKenna and Bell (*154*) noted a number of putative Late Cretaceous soricomorphs: *Otlestes*, *Paranyctoides*, and *Batodon*, but these have all been reinterpreted (see above) as basal Lipotyphla, or as basal to extant orders. Archibald (*190*) interpreted *Otlestes* as a basal eutherian, lacking derived characters of Lipotyphla, or any other modern order. Later, Averianov and Archibald (*191*) synonymized it with *Bobolestes* (from the same local fauna) and regarded it as a questionable zalambdalestoid. Archibald (*190*) was uncertain about *Paranyctoides* and *Batodon*, but retained these records as putative lipotyphlans pending discovery of further specimens, but we cannot confidently assert they are soricomorphs. Micropternodontids such as *Carnilestes* and *Prosarcodon* from the early Paleocene of Asia may be soricid relatives (*154*), but this has not been rigorously

tested. We tentatively regard Paleocene erinaceomorphs (*192*; see below) as the minimum constraint for a soricid–erinaceid clade. With records from the North American Torrejonian, the minimum estimate of *Adunator* can be tied to the top of the Danian at 61.5 Ma. For the soft maximum paleontological bound we suggest 131.5 Ma (see Archonta).

Laurasiatheria (minus Lipotyphla): bat–cow, sheep, dolphin, pig, horse, dog, cat (minimum = 62.5 Ma; soft maximum = 131.5 Ma)

The split between Chiroptera (bats) and its sister clade Ferungulata (Cetartiodactyla + Perissodactyla + Carnivora) depends on the oldest members of these included clades. The oldest artiodactyl is *Diacodexis* from the early Eocene of North America (c. 55 Ma). Artiodactyls are part of the larger clade Cetartiodactyla, with the Cetacea, whales and relatives, and these date back to the early Eocene as well, at about 53.5 Ma (*193*). The clade may also include the extinct mesonychids, which are known first from the Danian/Thanetian, some 62 Ma (*101*). The oldest carnivoramorphans are *Protictis* (above) and the miacoid *Ravenictis* from the Danian (Puercan, early Paleocene) of North America. Several carnivoran families radiated in the mid to late Paleocene of that continent (*194*). Undisputed perissodactyls do not appear until the early Eocene.

The oldest bats are *Archaeonycteris*, *Palaeochiropteryx*, and *Icaronycteris* (*101, 195*). *Icaronycteris* is reported first from the late Paleocene Clarkforkian Mammal Age, substage 3 (CF3), the *Phenacodus*/*Ectocion* acme zone, dated at 55.8–55.0 Ma (*196*). *Archaeonycteris* and *Palaeochiropteryx*, are marginally younger, coming from the MP7 level at Dormaal in Belgium and Rians, and possibly Meudon, in France. The European land mammal age MP7 is dated on the Paleocene/Eocene boundary, so 55.8 Ma ± 0.2 myr (*107*). Hence, neither bats nor cetartiodactyls have as old a fossil record as Carnivora, which dates to the early Torrejonian occurrence of *Protictis* (discussed earlier), for which we use the date 62.5 Ma.

The soft maximum constraint is again set at 131.5 Ma (see Archonta), given the lack of undisputed crown placentals throughout the Cretaceous.

Ferungulata: cow, sheep, dolphin, pig–horse, dog, cat (minimum = 62.5 Ma; soft maximum = 131.5 Ma)

The cow–dog split is equivalent to the branching point between the clades containing the Orders Cetartiodactyla (even-toed ungulates and whales) and Carnivora (flesh-eating placental mammals). This is synonymous with the clade Ferungulata, a clade within Laurasiatheria [as noted below this also includes Pholidota, following Waddell *et al.* (*111*)].

The oldest artiodactyl is *Diacodexis* from the early Eocene of North America (c. 55 Ma). Artiodactyls are part of the larger clade Cetartiodactyla, with the Cetacea, whales and relatives, and these date back to the early Eocene as well, at about 53.5 Ma (*197*). Mesonychids may be extinct relatives of cetartiodactyls, as they show conspicuous craniodental similarities with the latter, but lack the crucial pedal derived characters of cetartiodactyls (*198*). Mesonychids are known first from the Danian/Thanetian, some 62 Ma (*101*). The oldest carnivoramorphan is the miacoid *Ravenictis* from the Danian (Puercan, early Paleocene) of North America, and several carnivoran families radiated in the mid- to late Paleocene of that continent (*194*).

The clade Ferungulata includes also the Orders Perissodactyla and Pholidota, but neither of these dates back before the early Eocene. The oldest ferungulates are then Danian (Torrejonian, early Paleocene) in age, so the minimum age constraint for this clade is under 62.5 Ma. The soft maximum constraint is conservatively set at 131.5 Ma (see Archonta).

Zooamata: horse–cat, dog (minimum = 62.5 Ma; soft maximum = 131.5 Ma)

The dog–horse split is equivalent to the branching point between the Orders Carnivora and Perissodactyla [a clade that also encompasses pangolins in the Order Pholidota and was named Zooamata by Waddell and colleagues (*111*)]. The minimum age is determined from the oldest members of the carnivoran and perissodactyl lineages, as they predate records of Eocene pangolins (cf. *199*).

Flynn *et al.* (*200*) and others have modified the meaning of Carnivora so that it is restricted by them to the crown clade consisting of Caniformia + Feliformia. The wider clade traditionally called Carnivora, they term Carnivoramorpha. The oldest carnivoramorphans are the viverravids. The oldest generally accepted viverravid is *Protictis* from the Fort Union/Polecat Bench Formation, assigned to the basal Torrejonian (To1) NALMA, and dated as 63.6–62.5 Ma (*196*). If *Ravenictis* from Canada is also a carnivoramorphan (*201*), and that is debated (*202*), it extends this date back to at least the Puercan (Pu2), 65.4–64.3 Ma ± 0.3 myr. Most authors also agree that the extinct group Creodonta is the closest relative to Carnivoramorpha (*202*), and these date back to the Thanetian, 58.7–55.8 Ma ± 0.2 myr, younger than the oldest carnivormorphans.

The oldest perissodactyl is represented by fragmentary teeth that resemble the brontotheriid *Lambdotherium* from the late Paleocene site of Bayan Ulan in China (*203*), but the perissodacyl lineage may be extended further back in time. This places the dog–horse split minimally at the basal Torrejonian (62.5 Ma). As for the other supraordinal clades for which no fossil evidence exists up until the Paleocene, a paleontological soft maximum of 131.5 Ma (see Archonta) is tentatively suggested.

Carnivora: dog-cat (minimum = 39.68 Ma; soft maximum = 65.8 Ma)

The dog–cat split is equivalent to the branching point between the clades Caniformia (dogs, bears, raccoons, seals) and Feliformia (cats, mongooses, hyaenas), the major subdivisions of the Order Carnivora (*202*).

The oldest carnivorans are members of the Families "Miacidae" (paraphyletic) and Viverravidae, known from the early Paleocene onwards (*101*). However, these have recently been reconstructed outside the Caniformia–Feliformia clade (*202*), and so cannot provide a minimum date for the dog–cat split.

The oldest caniforms are amphicyonids such as *Daphoenus* and canids such as *Hesperocyon*, known first from the earliest Duchesnean North American Land Mammal Age (NALMA), which corresponds to magnetochron 18N, and is dated as 39.74 Ma ± 0.07 myr, based on radiometric dating of the LaPoint Tuff (*204*). *Tapocyon* may be an even older caniform; it comes from the middle Eocene, Uintan, dated as 46–43 Ma (*205*), although Flynn and Wesley-Hunt (*202*) place this taxon outside the Carnivora.

The oldest feliforms may be the nimravids, also known first from the White River carnivore fauna of the Chadronian NALMA, with uncertain records extending to the base of that unit (*206*). The earliest Chadronian corresponds to the top of magnetochron 17N, and an age of 37.2–36.7 Ma (*206, 207*).

Flynn *et al.* (*200*) suggest a caniform–feliform split around 50 Ma. Based strictly on the undisputed occurrence of caniforms from the Duchesnean, the minimum constraint from this event is at 39.68 Ma. The soft maximum constraint is based on the occurrence of the oldest stem carnivorans (miacids, viverravids) in the Torrejonian NALMA of the early Paleocene (see dog–horse), rounded down to the base of the Paleocene at 65.8 Ma.

Cetartiodactyla: pig-dolphin, cow, sheep (minimum = 52.4 Ma; soft maximum = 65.8 Ma)

The cow–pig split is equivalent to the major division in Artiodactyla between Ruminantia–Tylopoda, and "Suiformes," containing the controversial, but now fairly well-established hippo-whale clade (*208, 209*). The oldest terrestrial artiodactyls (e.g., *Diacodexis*) fall outside this clade. However, the oldest cetaceans, for example, early Eocene *Himalayacetus*, are not much younger. Because cetaceans comprise closer relatives to ruminant artiodactyls than do either suids or camels (*108*), their fossil record constrains the minimum divergence of crown artiodactyls.

Hence, the cow–pig division is dated minimally by the record of *Himalayacetus* from the base of the Subathu Formation in Pakistan (*210*) where it co-occurs with *Nummulites atacicus,* whose range correlates with nannoplankton zones 11–12, providing a minimum age of 52.4 Ma (*211*). The absence of any crown cetartiodactyls during the Paleocene may point to the soft maximum constraint at 65.8 Ma.

Whippomorpha-Ruminantia: dolphin-cow, sheep (minimum = 52.4 Ma; soft maximum = 65.8 Ma)

Within extant Cetartiodactyla, suiforms and camelids fall outside the whippomorph + ruminant clade [(*209*); "whippomorph" for cetaceans + hippopotamids was coined by Waddell *et al.* (*111*) and this name unfortunately appeared before more palatable alternatives such as Cetancodonta of Arnason *et al.* (*212*)]. However, the fossil cetacean *Himalayacetus* is both the oldest known whippomorph and crown cetartiodactyl, as older terrestrial artiodactyls (*Diacodexis*) cannot be unambiguously reconstructed within the crown clade (*208*). Hence, the minimum and soft maximum estimates for the last common ancestor of whippomorphs plus ruminants are the same as those for Cetartiodactyla already described.

Bovidae: cow-sheep (minimum = 18 Ma; soft maximum = 28.55 Ma)

The branching between the cow (*Bos*) and sheep (*Ovis*) is an intrafamilial split within the Family Bovidae. *Bos* is a member of the Tribe Bovini, and *Ovis* is a member of the Tribe Caprini, which belong respectively to the Subfamilies Bovinae and Antilopinae (*213*), although the monophyly of Antilopinae is questioned (*214*). These two subfamilies comprise the Family Bovidae, so the cow–sheep split corresponds to the point of origin of the crown Bovidae.

Fernandez and Vrba (*214*) point to a series of splits within Bovidae that gave rise to the major subfamilies 25.4–22.3 Ma, and they link this to a climatic change at the Oligocene/Miocene boundary. This date is, however,

not based directly on fossil evidence, but on a number of best-fitting dates from published morphological and molecular phylogenies.

A number of putative late Oligocene bovids (*101*) have since been rejected. The oldest putative bovid was *Palaeohypsodontus zinensis* from the Oligocene of the Bugti Hills, Balochistan, Pakistan, and the early Oligocene of Mongolia and China. This is a ruminant, but it lacks unequivocal anatomical features of Bovidae, and is currently excluded from that family (*215, 216*).

Fossil bovids may be identified in the fossil record by the presence of horn cores. The oldest such records, ascribed to *Eotragus*, come from the early Miocene of Western Europe and Pakistan. For example, *Eotragus noyi* from the base of the terrestrial sequence on the Potwar Plateau is dated at ~18.3 Ma (*217*).

Eotragus is attributed to Boselaphini, a tribe within the Subfamily Bovinae consisting of the nilgai and other four-horned antelopes. The oldest members of Antilopinae appear to come from the middle Miocene of three continents: *Caprotragoides* from Asia (India and Pakistan), *Tethytragus* from Europe (Spain and Turkey), and *Gentrytragus* from Africa (Kenya and Saudi Arabia), all dated at ~14 Ma (*218*). The oldest firmly dated bovid then places the minimum constraint on the origin of the family at 18.3 Ma, and we set the soft maximum constraint at the base of the late Oligocene (28.45 Ma ± 0.1 myr), encompassing the many equivocal stem bovids (*101*), so 28.55 Ma.

Placentalia: tenrec, elephant, armadillo–hedgehog, European shrew, bat, cow, sheep, dolphin, pig, horse, dog, cat, rabbit, pika, squirrel, guinea pig, mouse, rat, tree shrew, bushbaby, lemur, marmoset, macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 62.5 Ma; soft maximum = 131.5 Ma)

The human–tenrec split is equivalent to the origin of the clade comprising Boreoeutheria, Xenarthra, and Afrotheria. The oldest boreoeutherians are, as already noted (see, e.g., Zooamata), early Paleocene carnivorans, glires, or carpolestids, with the carnivorans at least 62.5 Ma. The oldest reported afrotherians are much younger, dating from the Eocene. The oldest are *Phosphatherium* and *Daouitherium* from Ypresian (lower Eocene) phosphorites of the Ouled Abdoun Basin of Morocco (*219*).

The minimum constraint for the Boreoeutheria/Xenarthra–Afrotheria split is then 62.5 Ma, and the soft maximum constraint is 131.5 Ma (see Archonta).

Atlantogenata: armadillo–tenrec, elephant (minimum = 55.6 Ma; soft maximum = 131.5 Ma)

As indicated later, the oldest undisputed afrotherians are Ypresian (basal Eocene) proboscideans from Morocco. The oldest xenarthrans (armadillos, anteaters, sloths) are slightly more ancient, known from the late Paleocene locality of Itaboraí in Brazil (*220*). Hence, the paleontological minimum divergence for this clade is in the mid- to upper Paleocene, corresponding to the upper Thanetian, 55.6 Ma. The soft maximum is provided on the same basis as for Archonta, hence 131.5 Ma.

Afrotheria: tenrec–elephant (minimum = 48.4 Ma; soft maximum = 131.5 Ma)

The tenrec–elephant split represents a deep division within Afrotheria. According to current phylogenies, the tenrec, golden moles (Chrysochloridae), elephant shrews (Macroscelidea), and aardvark (Tubulidentata) may form one clade within Afrotheria, and the elephants, hyraxes and sirenians form the other, termed Paenungulata. Paenungulata is widely accepted as a valid clade, having been established on morphological characters, and now confirmed by molecular analyses. In any case, the last common ancestor of tenrec and elephant corresponds to the base of crown-clade Afrotheria.

The oldest fossil aardvarks, tenrecs, golden moles, and elephant shrews are generally stated to be Miocene (*154*), with a possible older elephant shrew, *Metoldobotes* from the late Eocene Jebel Qatrani Formation of Egypt. Tabuce *et al.* (*221*) report a much older golden mole, *Chambius*, from the Chambi locality in Tunisia (Ypresian). Seiffert and Simons (*222*) tentatively suggested that *Widanelfarasia* from near the Eocene/Oligocene boundary in Egypt may be the closest relative to a tenrec–golden moles clade. These records are equaled or predated by the oldest paenungulates. Zack *et al.* (*223*) argue that Paleocene apheliscines from North America share a close evolutionary relationship with elephant shrews. The oldest hyraxes are known from the Eocene of North Africa (*219, 221*). The oldest sirenians are *Prorastomus* and *Pezosiren* from the early middle Eocene of Jamaica (*219*). The oldest proboscidean fossils are *Phosphatherium* and *Daouitherium* from Ypresian (lower Eocene) phosphorites of the Ouled Abdoun Basin of Morocco (*219*). This basal age is confirmed by reports of early Eocene (Ypresian) hyrax and proboscidean fossils from the Tamaguélelt Formation of Mali (*224*). Extinct putative outgroups of crown-group Paenungulata, such as Desmostylia and Embrithopoda (*Arsinoitherium*) are younger, being Oligocene in age, while the Anthracobunidae date back to the early Eocene.

At present, no extant clade within Afrotheria, nor any confirmed extinct afrothere clade (with the apheliscine/"condylarth" possibility deserving further scrutiny), predates the Ypresian (early Eocene), dated at ca. 54 Ma, with a minimal age corresponding to the upper limit of the Ypresian at 48.4 Ma. Further study might reveal that certain Paleocene groups belong within one or other afrothere branch, and that could increase the minimum age constraint. The soft maximum constraint is 131.5 Ma (see Archonta).

Theria: opossum, kangaroo–tenrec, elephant, armadillo, hedgehog, European shrew, bat, cow, sheep, dolphin, pig, horse, dog, cat, rabbit, pika, squirrel, guinea pig, mouse, rat, tree shrew, bushbaby, lemur, marmoset, macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 124 Ma; soft maximum = 171.2 Ma)

The human–opossum branching point is of course synonymous with the split of marsupials and placentals. The earliest alleged "marsupial" dental fossils come from the mid-Cretaceous of North America, including *Kokopellia juddi* reported (*225*) from the Mussentuchit Member, in the upper part of the Cedar Mountain Formation, Utah, which is dated as middle to late Albian on the basis of bivalves and palynomorphs. A date of 98.37 Ma ± 0.07 myr was obtained from radiometric dating of zircons in a bentonitic clay layer. However *Kokopellia* has not been demonstrably placed within crown Marsupialia by any phylogenetic analysis, and indeed lies outside the marsupial crown (but still on its stem) in the few cases in which it has been tested (e.g., *226*). Even older is the boreosphenidan *Sinodelphys szalayi* from the Yixian Formation, Liaoning Province, China, which is placed phylogenetically closer to marsupials than to placentals by Luo *et al.* (*160*). This then has taken the stem of the marsupial clade back to the Barremian with an age of at least 124 Ma.

The oldest eutherians (on the stem to Placentalia) were also, until recently, restricted to the mid- and late Cretaceous (*101*), but subsequent finds have pushed the age back step-by-step deeper into the Early Cretaceous. First were *Prokennalestes trofimovi* and *Prokennalestes minor*, from the Höövör beds of Mongolia (*227*), dated as either Aptian or Albian. Then came *Montanalestes keeblerorum* (*228*) from the Cloverly Formation (late Aptian–early Albian, ~100 Ma). Then, *Murtoilestes abramovi* was named (*229*) from the Murtoi Svita, Buryatia, Transbaikalia, Russia, being dated as late Barremian to middle Aptian (say, 128–120 Ma). These three taxa were based on isolated jaws and teeth. These

were all topped by the spectacular find of *Eomaia scansoria* in the Yixian Formation of Liaoning Province, China (*159*), a complete skeleton with hair and soft parts preserved. Dating of the Jehol Group of China has been contentious, with early suggestions of a Late Jurassic age for some or all of the fossiliferous beds. Biostratigraphic evidence now confirms an Early Cretaceous (Barremian) age, with several radiometric dates, using different techniques, on three tuff layers that occur among the fossil beds of 124.6 Ma ± 0.01 myr, 125.06 Ma ± 0.18 myr, 125.2 Ma ± 0.9 myr (*162*). This gives an encompassing age designation of 125.0 Ma ± 0.4 myr for the span of the three tuff layers, and for the fossiliferous beds of the Yixian Formation, based on direct dating. Thus, we conclude a minimum constraint of ca. 124.6 Ma for the divergence of marsupial and placental mammals. An alternative view (*230*) places southern tribosphenic taxa (see later) on the stem to Theria, pushing the minimum age for Theria to the Jurassic in order to include such taxa as *Ambondro*.

Given the proposal of Woodburne *et al.* (*230*) that southern, tribosphenic mammals such as *Ambondro* are therian (even eutherian), we would set the soft maximum age constraint for Theria within the Jurassic (Bathonian) at 167.7 Ma ± 3.5 myr, so 171.2 Ma.

Marsupialia: opossum–kangaroo (minimum = 61.5 Ma; soft maximum = 131.5 Ma)

The opossum–kangaroo split is equivalent to the divergence of the two main crown marsupial clades: Ameridelphia and Australidelphia. There are older metatherians from the Cretaceous, such as *Kokopellia* and *Sinodelphys*, but these fall phylogenetically outside of the marsupial crown radiation (e.g., *226*). Indeed, non-marsupial metatherians persist well into the Tertiary and, despite close dental similarities with crown didelphids (e.g., *Herpetotherium*), they fall outside Marsupialia when their full anatomical diversity is examined in a cladistic context (*231*). Hence, the oldest marsupials are from the Tiupampa fauna of Bolivia (*232*), dated close to 63 Ma and minimally corresponding to the top of the Danian at 61.7 Ma (*107*). Specifically, the Tiupampan genus *Khasia* has been reconstructed as having a particularly close relationship to microbiotheres (*233*), a radiation for which the only living representatives are two species of *Dromiciops*. Importantly, microbiotheres have been phylogenetically linked not with other South American marsupials, but with the Australidelphia, based on both morphological and molecular data (e.g., *231*, *234*). The oldest australidelphians from Australia

include possible bandicoots and/or microbiotheres from the early Eocene locality of Murgon (*235*). Other elements of the Tiupampa fauna, including the skeletally well-known *Pucadelphys* and *Mayulestes*, are not demonstrably part of crown Marsupialia (*231*), nor is the early Paleocene taxon *Cocatherium* from Chubut province, Argentina (*233*). Hence, the paleontological minimum constraint for Marsupialia is late Paleocene, 61.5 Ma.

As noted earlier, numerous metatherian remains are known from the Cretaceous of both North America (*Kokopellia*; cf. *225, 228*) and Mongolia (*Asiatherium*; cf. *236*), with the Early Cretaceous *S. szalayi* being the oldest undisputed metatherian (*160*). Hence, we place the soft maximum constraint for Marsupialia at the Liaoning beds that produced *Sinodelphys*, at 131.5 Ma.

Mammalia: platypus–opossum, kangaroo, tenrec, elephant, armadillo, hedgehog, European shrew, bat, cow, sheep, dolphin, pig, horse, dog, cat, rabbit, pika, squirrel, guinea pig, mouse, rat, tree shrew, bushbaby, lemur, marmoset, macaque, gibbon, orangutan, chimp, neanderthal, human (minimum = 162.9 Ma; soft maximum = 191.1 Ma)

The base of the crown clade of modern mammals, marking the split between Monotremata, represented by the platypus, and Theria, represented by the human, might have a number of positions, depending on how many of the extinct Mesozoic mammal groups are included in the clade.

As noted earlier, the oldest marsupial, *Sinodelphys*, and the oldest placental, *Eomaia*, take the age of Theria back to about 125 Ma. *Vincelestes* from the La Amarga Formation of Argentina is dated as Hauterivian, and shows the existence of stem Theria at least ca. 136 Ma.

According to a widely accepted cladogram of Mesozoic mammals (*160, 237, 238*), the Theria are part of a larger clade Theriimorpha that includes further extinct clades: Triconodonta, Multituberculata, Symmetrodonta, and Dryolestoidea. Most of these originated in the Late Jurassic, but triconodonts and dryolestoids began earlier, in the Middle Jurassic. Basal triconodonts include *Amphilestes* and *Phascolotherium* from the Stonesfield Slate, referred to the *Procerites progracilis* Zone of the lower part of the middle Bathonian stage on the basis of ammonites (*239*), and so dated as 166.9–166.5 Ma ± 4.0 myr (*240*). Tooth-based mammal taxa from the Early Jurassic of India (*Kotatherium, Nakundon*) and North America (*Amphidon*) that have been ascribed to Symmetrodonta (*238*), are not convincingly members of the clade (*241*), and so are ignored here. The oldest

dryolestoid appears to be *Amphitherium*, also from the Stonesfield Slate.

The oldest monotremes are *Steropodon* and *Kollikodon* from the Griman Creek Formation, Lightning Ridge, South Australia, and dated as middle to late Albian, 109–100 Ma. *Teinolophos* is from the Wonthaggi Formation, Flat Rocks, Victoria, and is dated as early Aptian, 125–121 Ma. In the new cladistic view (*160, 226, 237, 238*), the Ausktribosphenida from Gondwana are the closest relatives of Monotremata, forming together the Australosphenida. Oldest are *Asfaltomylos* from the late Middle Jurassic (Callovian) Cañadon Asfalto Formation of Cerro Condor, Argentina (*242*) and *Ambondro* from the upper part of the Isalo "Group" (Middle Jurassic, Bathonian) of Madagascar (*243*). The position of the Madagascar find in the Bathonian is uncertain, so the age range is 167.7 Ma ± 3.5 myr–164.6 Ma ± 4.0 myr. The human–platypus split is then dated on the oldest theriimorph from 166.9–166.5 Ma ± 4.0 myr, similar in age to the less well-dated oldest australosphenidan. On the basis of the available evidence, we follow Luo and colleagues and accept a minimum constraint of 162.9 Ma.

The closest relative of Australosphenida + Theriimorpha is Docodonta, and the oldest docodonts are from the Bathonian of Europe, with a possible earlier form from the Kota Formation of India. Further outgroups, Morganucodontidae, *Sinoconodon*, and *Adelobasileus*, are known from the Late Triassic and Early Jurassic. The Kota Formation, and several other units from other parts of the world that have yielded fossil mammals, but nothing assignable to the Australosphenida or Theriimorpha, date to the later half of the Early Jurassic, equivalent to the Pliensbachian and Toarcian stages (189.6 Ma ± 1.5 myr–175.6 Ma ± 2.0 myr), and so 191.1 Ma should be used as a soft maximum constraint.

Amniota: bird–mammal (minimum = 312.3 Ma; soft maximum = 330.4 Ma)

The ultimate divergence date between birds and mammals has been quoted many times as 310 Ma, generally tracing back to Benton (*32*). Van Tuinen and Hadly (*244*) trace the history of the use of this date in molecular analyses, and they quote a range of estimates from 338 to 247 Ma, with a preference for the 310 Ma date on the basis of reassessment of the Late Carboniferous timescale.

This estimate has been criticized for being used without error bars (*36, 244*), for being based on uncertain fossils and hence too old (*34*), for being misdated (*37, 244*), and

for being poorly bracketed by outgroups above and below (*37*). Reisz and Müller (*37, 54*) indeed argue that this calibration point should no longer be used largely because its soft maximum bound is so poorly constrained.

The ultimate ancestor of birds and mammals has to be tracked back to the base of the Synapsida and Sauropsida, the larger clades that include mammals and birds, respectively. These two clades together make up Amniota, the clade containing all tetrapods other than amphibians, and the relationships of major groups is agreed by most (e.g., *244–247*). The question of the ultimate bird–mammal split becomes synonymous then with dating the origin of the clade Amniota.

The oldest identified synapsid is *Protoclepsydrops* from the Joggins Formation of Joggins, Nova Scotia. The age of the Joggins Formation has been much debated, and figures in the range from 320 to 305 Ma have been cited recently. Reisz and Müller (*37*) indicate an age of 316–313 Ma, while Van Tuinen and Hadly (*244*) settle for 310.7 Ma ± 8.5 myr. Detailed field logging and biostratigraphy (*248–250*) confirm that the Joggins Formation falls entirely within the Langsettian European time unit, equivalent to the Westphalian A, and roughly matching the Russian Cheremshanian, in the later part of the Bashkirian Stage. Earlier dates for these units were equivocal (*251*), but the Langsettian is given as 314.5–313.4 Ma ± 1.1 myr in GTS2004 (*252*).

Protoclepsydrops has been classed as an ophiacodontid, not a member of the basalmost synapsid families—Eothyrididae, Caseidae, or Varanopseidae—whose basal members, if ever found, might be of the same age or older. *Protoclepsydrops haplous* is known from one incomplete partial skeleton and skull (*253*), but the remains are fragmentary; even the identification of these remains as a synapsid has been questioned (*254, 255*). Lee (*34*) used this uncertainty to reject *Protoclepsydrops* as informative in this discussion, and to look at the next oldest synapsids, such as *Echinerpeton* and *Archaeothyris* from the Morien Group of Florence, Nova Scotia (Myachkovian, upper Moscovian, 307.2–306.5 Ma). Because each retained only one derived character of Synapsida, Lee (*34*) rejected them, and moved up to more complete material of basal synapsids from some 288 Ma. Van Tuinen and Hadly (*244*) rejected *Protoclepsydrops* as a useful marker of the bird–mammal split, but accepted *Archeothyris* as reasonable, with a date of 306.1 Ma ± 8.5 myr.

Phylogenetically, the basalmost member of the amniote branch, the Sauropsida (sometimes called Eureptilia, or Reptilia) is *Coelostegus* from the Upper Carboniferous (Moscovian, Myachkovskian; 306.5 Ma ± 1 myr) of the Czech Republic (*256*). This is not, however, the oldest sauropsid. Basal sauropsids include a number of genera formerly assigned to the paraphyletic "Protorothyrididae," outgroups to Diapsida, and the oldest of these in *Hylonomus*, also from the Joggins Formation at Joggins, Nova Scotia (*257–259*). Within Sauropsida, the sister clade to Diapsida + "Protorothyrididae" is the clade Captorhinidae, but the oldest captorhinid is younger—*Romeria primus* from the Moran Formation of Texas (Early Permian, Sakmarian, ~294–284 Ma; *260*). Lee (*34*) cast doubt on the assignment on *Hylonomus* to the sauropsid clade, and preferred to redate that branch also to some 288 Ma.

Lee's (*34*) proposal would move the mammal–bird split date from somewhere around 310 to 290 Ma, whereas Van Tuinen and Hadly (*244*) settled for 305 Ma as a minimal date. However, Reisz and Müller (*37*) and Van Tuinen and Hadly (*244*) suggested that Lee was wrong to cast doubt on nearly all the Carboniferous synapsids and sauropsids—many are diagnostic of one or other group. More importantly though, Reisz and Müller (*37*) pointed out that the question of dating the ultimate bird–mammal split is synonymous with dating the origin of Amniota. So, it may be uncertain whether *Protoclepsydrops* is a synapsid, and *Hylonomus* is not a diapsid, and the "Protorothyrididae" are clearly paraphyletic (*256*), but all these taxa are diagnostically members of Amniota, so the origin of Amniota happened before the age of the Joggins Formation of Nova Scotia.

Older evidence of amniotes has been reported by Falcon-Lang *et al.* (*261*), footprints with a number of amniote derived characters (pentadactyl manus and pes, slender digits whose relative lengths approximate a phalangeal formula of 23453 (manus) and 23454 (pes), narrow digit splay (40–63°), putative transverse scale impressions on digit pads, and straight tail drag) that come from the Grand Anse Formation of Nova Scotia. This unit lies 1 km below the Joggins Formation, and is dated at ~1 myr older than the Joggins. However, although we are convinced the footprint evidence represents amniotes, such a fossil is less reliable than a skeletal body fossil, and can be taken as an indication of a future increase in the minimum constraint on the bird–mammal split.

The minimum constraint on the mammal–bird split, equivalent to the minimum age of the origin of clade Amniota corresponds to the age of the Joggins Formation. This is 314.5–313.4 Ma ± 1.1 myr, a date based on

biostratigraphy (palynology) and exact dating from elsewhere, conferring a minimum constraint of 312.3 Ma.

The soft maximum constraint on the bird–mammal split is based on the next richly fossiliferous units lying below these horizons. The first is the East Kirkton locality, source of a diverse fauna of batrachomorphs and reptiliomorphs (see human–toad split later), but that has hitherto not yielded anything that could be called either a diapsid or a synapsid. Further fossiliferous sites of similar facies lie below the East Kirkton level, and they have not yielded reptile remains. We take the age of the fossiliferous Little Cliff Shale of the East Kirkton locality (Brigantian; 328.8–326.4 Ma ± 1.6 myr) as the basis for the soft maximum age constraint of 330.4 Ma.

Diapsida: lizard–crocodile, emu, chicken, zebrafinch (minimum = 255.9 Ma; soft maximum = 299.8 Ma)

The clades Crocodylia (modern crocodilians and extinct relatives) and Squamata (modern lizards and snakes and their extinct relatives) are members, respectively, of the larger clades Archosauromorpha and Lepidosauromorpha. The ultimate split between crocodilians and lizards then is marked by the split between those two, and they, together with a number of basal outgroups, form the major clade Diapsida. Through a series of cladistic analyses (*245, 257, 258, 262–265*), the topology of the basal region of the cladogram around the split of Archosauromorpha and Lepidosauromorpha has been agreed (although some higher parts of the cladogram are still much debated, especially the placement of Sauropterygia and Ichthyosauria).

The most ancient archosauromorph is the "prolacertiform" *Protorosaurus speneri* from the Kupferschiefer of Germany and the Marl Slate of NE England (*266*). Both geological units are correlated with each other on independent geological evidence, and defined as the basal unit of the Zechstein 1 (EZ1; Werra Folge) depositional cycle. The two units were generally assigned to the Kazanian (e.g., *260*, p. 695), but subsequent stratigraphic revisions have shown that the Zechstein falls above the Illawarra Reversal, which is at the Wordian–Capitanian boundary, and the Zechstein I contains fossils characteristic of the Capitanian (*267*). It is unclear how much of the Capitanian is represented by the Zechstein, but it probably represents the upper part, so 263.8–260.4 Ma ± 0.7 myr. Roscher and Schneider (*268*) estimate a minimum age for the Kupferschiefer as 255.9 Ma, so we accept that here, as a younger estimate, based on new dating

evidence, than the date of 259.7 Ma given by Benton and Donoghue (*3*).

The most ancient lepidosauromorph is debated—Benton (*260*, p. 688) indicated that *Saurosternon bainii*, sole representative of the Saurosternidae, may be the oldest, but he was uncertain. Other authors (*246, 257, 269*) were more convinced that this is a true lepidosauromorph. The doubt arises because the taxon is based on a single partial skeleton lacking the skull. *Saurosternon* is from the *Cistecephalus* or *Dicynodon* Assemblage Zone of South Africa (*270*) equivalent to the uppermost Wuchiapingian or Changhsingian, respectively, perhaps some 257–251 Ma. However, numerical cladistic analyses (*271, 272*) have shown unequivocally that *Saurosternon* and other supposed Permo-Triassic "lizards" are not lepidosauromorphs or even neodiapsids. If *Saurosternon* is not a lepidosauromorph, the next possibility would be a sauropterygian. The uncertainty here is whether sauropterygians are lepidosauromorphs—the group was unplaced phylogenetically for some time, but deBraga and Rieppel (*245*) and others have made a strong case that these marine reptiles are unequivocal lepidosauromorphs. Benton (*260*, p. 70) listed two Lower Triassic (Scythian) sauropterygians, *Corosaurus* and *Placodus*, but the dating of both is uncertain. *Corosaurus* is from the Alcova Limestone Member of the Chugwater Formation in Wyoming, formerly assigned to the Middle or Upper Triassic, but noted as Lower Triassic by Storrs (*273*). The precise age is hard to pin down. The Lower Triassic *Placodus* is from the Obere Buntsandstein of Pfalz, Germany, a unit dated as spanning the Olenekian–Anisian boundary, and ranging in age from 246–244 Ma ± 1.5 myr.

Based on the oldest neodiapsid, *Protorosaurus*, the minimum constraint on the divergence of crocodilians and lizards is 255.9 Ma.

In order to establish the soft maximum constraint on this divergence, outgroups to Neodiapsida are considered. Ichthyosauria are known first in the Early Triassic, younger than the minimum age constraint. Younginiformes, Weigeltisauridae (*Coelurosauravus*), and *Claudiosaurus* are of similar age to *Protorosaurus*, or younger. Next oldest is *Apsisaurus* from the Archer City Formation of Texas, dated as Asselian (299–294.6 Ma ± 0.8 myr) (*260*), and so 299.8 Ma, although its diapsid affinities have been questioned (*274*). This is a long way below the minimum age constraint, but there is a well-known "gap" in suitable fossiliferous formations through the mid-Permian, and we retain this possibly exaggerated soft maximum constraint.

*Archosauria: crocodile–emu, chicken, zebrafinch
(minimum = 239 Ma; soft maximum = 250.4 Ma)*

The most recent common ancestor of crocodilians and birds was an archosaur that lay at the deep junction of the two major clades within Archosauria: Avemetatarsalia/Ornithodira, the "bird" line and Crurotarsi, the "crocodile" line (*275–277*). These two clades together form the Avesuchia (= "crown-group Archosauria").

The basal crurotarsans are the poposaurid *Bromsgroveia* from the Bromsgrove Sandstone Formation of England, and the "rauisuchians" *Wangisuchus* and *Fenhosuchus* from the Er-Ma-Ying Series of China, *Vjushkovisaurus* from the Donguz Svita of Russia, the ctenosauriscid *Arizonasaurus* from the Moenkopi Formation, and *Stagonosuchus* and "*Mandasuchus*" from the Manda Formation of Tanzania (*260*). All these records are dated as Anisian, but most cannot be dated more precisely; *Arizonasaurus* though can be assigned to the lower Anisian (*278*). This gives an age range of 245 Ma ± 1.5 myr–241 Ma ± 2.0 myr.

The basal avemetatarsalian is *Scleromochlus* from the Carnian of Scotland, but older relatives are *Marasuchus*, *Lagerpeton*, and *Pseudolagosuchus* from the Chañares Formation of Argentina, dated as Ladinian, so 237 Ma ± 2.0 myr–228.0 Ma ± 2.0 myr.

The minimum constraint on the divergence date for birds and crocodiles then falls at the top of the lower Anisian (245 Ma ± 1.5 myr–241 Ma ± 2.0 myr), and so 239 Ma, an increase of 4 myr over the date given by Benton and Donoghue (*3*) as a result of the closer dating of *Arizonasaurus*.

The soft maximum constraint may be assessed from the age distribution of immediate outgroups to Avesuchia, the Proterochampsidae, Euparkeriidae, Erythrosuchidae, and Proterosuchidae (*275–277*). Numerous fossil sites from around the world in the Olenekian, the stage below the Anisian, have produced representatives of these outgroups, but not of avesuchians, and so the Olenekian (249.7 Ma ± 0.7 myr–245 Ma ± 1.5 myr) marks the soft maximum age constraint, and so 250.4 Ma.

Neornithes: emu–chicken, zebrafinch (minimum = 66 Ma; soft maximum = 86.5 Ma)

The divergence of emu and chicken is synonymous with the deep divergence between the Palaeognathae (ratites, or flightless birds) and the Neognathae (all other, flying, birds).

The oldest palaeognaths are the lithornithids, a family known from the Paleocene and Eocene of North America. A putative latest Cretaceous lithornithid was reported by Parris and Hope (*279*) from the New Jersey greensands. The age of these deposits has been much debated (*280*), and they fall either below or above the K-T boundary (65.5 Ma ± 0.3 myr). An older specimen might be mistakenly assigned here: the pelvis of a large flightless bird, *Gargantuavis philoinos*, reported (*281*) from the base of the Marnes de la Maurines Formation, in association with dinosaurs of late Campanian to early Maastrichtian aspect. These authors were clear that *Gargantuavis* was not a palaeognath, and suggested it might be related to the non-neornithine *Patagopteryx*.

The oldest confirmed neognath fossil is the anseriform *Vegavis* from 66 Ma, and this has to be the minimum constraint on the divergence date for palaeognaths and neognaths. The soft maximum constraint is currently the same as for the chicken–zebrafinch split below, namely the clades Ichthyornithiformes and Hesperornithiformes of the Niobrara Chalk Formation, dated as Santonian (85.8–83.5 Ma ± 0.7 myr), and so 86.5 Ma.

Neognathae: chicken–zebrafinch (minimum = 66 Ma; soft maximum = 86.5 Ma)

The phylogeny of major groups of modern flying birds (clade Neognathae) has been hard to resolve. Recent morphological and molecular analyses now agree on a deep divergence between the clade Galloanserae, comprising Galliformes (chickens and game birds) and Anseriformes (ducks) on the one hand, and Neoaves (all other flying birds) on the other (*280, 282*).

The oldest purported galloanserine is *Teviornis gobiensis*, a presbyornithid anseriform from the Gurilyn Tsav locality of Mongolia (*283*). Sediments here come from the lower portion of the Nemegt Horizon, at the base of the Nemegt Formation. The Nemegt Formation is assigned to the early Maastrichtian (*284*), dated as 70.6 Ma ± 0.6 myr to 69.6 Ma ± 0.6 myr. Doubt has been cast, however (*285*), on whether *Teviornis* is a neognath, let alone a galloanserine, so the next youngest purported neognath should be selected until this issue is clarified. A further latest Cretaceous anseriform is *Vegavis iaai* from lithostratigraphic unit K3 of Vega Island, Antarctica, dated as mid- to late Maastrichtian, ~68–66 Ma (*286*). The oldest galliform fossil that can be identified with confidence is much younger, early Eocene (*282*).

The oldest neoavian is debated, with dozens of records of gaviiforms, pelecaniforms, charadriiforms, procellariiforms, and psittaciforms from the latest Cretaceous (most are close to the Cretaceous–Tertiary boundary, 65.5 Ma; *287, 288*). The most complete fossil is *Polarornis gregorii*, described as a loon (gaviiform) from the Lopez

de Bertodano Formation of Seymour Island, Antarctica (*289*). This stratigraphic unit is dated as mid- to late Maastrichtian on the basis of microplankton (*290*), so 69.6–65.5 Ma ± 0.3 myr. Dyke and Van Tuinen (*280*) indicate some doubt about the taxonomic assignment of the specimen and about its geological provenance.

Even if the various neoavian specimens fall close to the Maastrichtian–Danian boundary, and if there is some doubt about *Polarornis* and *Teviornis*, the galloan-serine record of *Vegavis* is older, and dates the minimum constraint on chicken–zebrafinch divergence at 66 Ma, on the basis of biostratigraphy and indirect dating.

The soft maximum constraint is based on older bird-bearing deposits that match some at least of the facies represented in the late Maastrichtian, which are broadly from the shallow marine to coastal belt. Fossil birds, most notably, hundreds of specimens of *Hesperornis*, *Baptornis*, and *Ichthyornis* (members of the clades Ichthyornithiformes and Hesperornithiformes, both outgroups to Neornithes), but no Neornithes have been found in abundance from the Niobrara Chalk Formation of Kansas and neighboring states, dated as Santonian (85.8–83.5 Ma ± 0.7 myr), and so 86.5 Ma.

Tetrapoda: toad–bird, mammal (minimum = 330.4 Ma; soft maximum = 350.1 Ma)

The African clawed toad (*Xenopus laevis*) is a representative of modern Amphibia (the clade Lissamphibia, including frogs and toads, salamanders, and caecilians), and the human–toad split is equivalent to the deep branching point between Amphibia and Amniota. Within crown Tetrapoda, this is the split of Batrachomorpha (extant lissamphibians and extinct relatives) and Reptiliomorpha (extant amniotes and their extinct relatives).

The oldest batrachomorph is *Balanerpeton woodi*, a basal temnospondyl from the East Kirkton locality in Scotland. Another putative basal batrachomorph is *Eucritta melanolimnetes*, from the same location, described as a possible baphetid (*291*), but possibly a batrachomorph (*292*). The fossils come from the Little Cliff Shale, a unit within the East Kirkton Limestone, a subdivision of the upper Oilshale Group of the Midland Valley of Scotland. The fossil beds are ascribed to the Brigantian (D2; lower portion) of the Viséan stage, based on biostratigraphic comparisons of the fossil plants, pollen, and bivalves with the rich records of Lower Carboniferous sites throughout Europe (*293*). The Brigantian regional stage is dated 328.8–326.4 Ma ± 1.6 myr.

The oldest reptiliomorphs are the basal lepospondyl *Westlothiana lizziae*, and the aïstopod *Lethiscus*

stocki (*292*). *Westlothiana* and *Lethiscus* are both from the Viséan. *Westlothiana* comes from the East Kirkton locality, and is dated at 327.6 Ma ± 2.8 myr (see earlier). *L. stocki* is from the Wardie Shales, part of the Lower Oil Shale Group, near Edinburgh, and dated as older than the East Kirkton locality (*293*). The Wardie Shales are assigned to the Holkerian regional stage on the basis of fossil fishes and palynomorphs (*294*), dated as 339.2–332.4 Ma ± 2.0 myr.

Van Tuinen and Hadly (*244*) reviewed the amphibian–amniote divergence date in detail, but assigned the Wardie Shales to the Asbian, the stage above the Holkerian, and so came to an age of 332.3 Ma. Further, they used radiometric dates from Menning *et al.* (*251*) which have been revised in GTS2004 (*267*). Our minimum constraint on the human–toad divergence is 330.4 Ma, based on *Lethiscus*, and biostratigraphic placement of the Wardie Shales Formation, with radiometric dating of the Holkerian from elsewhere.

The soft maximum constraint is harder to determine because most of the close outgroups to the batrachomorph–reptiliomorph clade are known only from younger deposits: the oldest baphetids and crassigyrinids are from the Brigantian (*260*), the oldest colosteids from the Asbian (*260*). The whatcheeriids *Whatcheeria* and *Pederpes*, from North America and Europe, respectively, are older, however, and dated to the Ivorean regional North American stage, and so 348–345.3 Ma ± 2.1 myr. These horizons are underlain by further units of Famennian age, dated as 374.5 Ma ± 2.6 myr–359.2 Ma ± 2.5 myr, with basal tetrapods known from several continents, but no batrachomorphs or reptiliomorphs. We choose the whatcheeriids as marking the soft maximum constraint, even though they are phylogenetically more distant from crown Tetrapoda than baphetids and colosteids—but the latter two are younger than *Lethiscus*. Thus, we propose a date of 350.1 Ma as a soft maximum constraint.

Osteichthyes: zebrafish, Medaka, stickleback, Takifugu, Tetraodon–toad, bird, mammal (minimum = 416.0 Ma; soft maximum = 421.75 Ma)

This divergence event represents the origin of crown Osteichthyes and the splitting of Actinopterygii and Sarcopterygii. Thus, the minimum constraint depends on determining the oldest member of either clade.

The earliest representative of total-group Actinopterygii may be *Andreolepis hedei*, known from microfragments from Gotland, Sweden (*295–298*), and elsewhere (*299*). It has been assigned to total-group Actinopterygii

on the following derived characters: rhomboid scale shape, ganoine-covered scales. The oldest occurrence that is readily constrained is from the lower part of division C of the Hemse Marl at Västlaus, Gotland, Sweden (*296*). Although there are no direct radiometric dates from the Ludlow of Gotland, these sections have been incorporated into a graphic correlation composite standard that includes radiometric dates (*300, 301*). Thus, a date for this occurrence can be established from the composite standard through the line of correlation, which equates to 421.75 Ma.

The certainty with which *A. hedei* is assigned to Actinopterygii is obviously less than it might be were it known from articulated remains. It is known from a number of skeletal elements (*295, 302–304*), rather than mere scales, as are the other, slightly younger, early records of Actinopterygii (*305, 306*), but these have led some researchers to conclude a stem-Osteichthyes, rather than a stem-Actinopterygii affinity (*304*).

Naxilepis, although known only from scales (*306*), possesses a further derived character of total-group Actinopterygii, in addition to those exhibited by *A. hedei*, namely a narrow-based dorsal peg and discrete rows of ganoine. The earliest occurrence is from the Miaogao Member of the Cuifengshan Formation of Quijing District, Yunnan, China, where it co-occurs with the conodont *Ozarkodina crispa* (*306, 307*), although this has not been substantiated. As later, this constrains the age of the first occurrence of *Naxilepis* between the middle Ludlow and the Ludlow–Pridoli boundary (418.7 Ma).

The earliest macroremains assignable to total group Actinopterygii are of *Dialipina markae* from the Lochkovian of Siberia (*308*), which is also known from fully articulated remains from the Lower Devonian (Emsian) of the Canadian Arctic (*309*). Justification for the Lochkovian age assignment is not clear (*310*).

The earliest record of the sarcopterygian total group is *Psarolepis romeri*, known (in stratigraphic order) from the Yulongsi (*311*), Xishancun (*311*), and Xitun (*312*) members of the Cuifengshan Formation, Quijing District, eastern Yunnan, China (the recently described *Meemania eos* is apparently a more basal member of the sarcopterygian stem but it is known only from the Xitun Member; *313*). The dating of these occurrences relies primarily upon biostratigraphic dating of a lithostratigraphic correlation of the Yulongsi Member in neighboring Guangxi, where the conodont *O. crispa* has been found in the middle of the Yulongsi Member (*314*). The lower limit of the stratigraphic range of *O. crispa* is constrained by the Ludlow–Pridoli Boundary (*315*), which

has been dated as 418.7 Ma ± 2.7 myr (*316*). In the type Ludlow Series the upper range limit on *O. crispa* is just a few meters below its lower limit (*315*) (the latest Ludlow and earliest Pridoli are probably unrepresented in the Ludlow type area; *317*). Although it is difficult to provide a direct date on this horizon, zircons from a bentonite 12 m deeper in the Ludlow type section have provided a U-Pb Zircon age of 420.2 Ma ± 3.9 myr (*318*). There is a report of *O. crispa* as low as "middle Ludlow" (*319*), although this is just one of a number of possible interpretations of the conflicting biostratigraphic data. Attempts to directly date the Quijing succession biostratigraphically have yielded the conodont *Oulodus elegans detorta* from the upper part of the Yulongsi Member (*320*). The stratigraphic range of *O. elegans detorta* is confined to its zone, which is the ultimate conodont zone of the Silurian (*321*). Thus, direct and indirect biostratigraphic dating is in agreement concerning the age of the middle and upper parts of the Yulongsi Member, indicating that the earliest record of *Psarolepis* is no younger than latest Ludlow (418.7 Ma ± 2.7 myr) and possibly older than 420.2 Ma ± 3.9 myr.

Although originally described as a sarcopterygian (*311, 312*), *Psarolepis* has also been interpreted as stem-Osteichthyes (*322, 323*). However, more recent and universal analyses have confirmed its assignment to the sarcopterygian stem-lineage (*313, 324*).

After *Psarolepis*, the next oldest representatives of total-group Sarcopterygii, *Diabolepis*, *Youngolepis*, and *Achoania* are approximately coeval. They come from the Xishancun Member of the Cuifengshan Formation of Qujing District. The Xishancun Member is clearly younger than the underlying Yulongsi Formation, the upper part of which is dated as latest Silurian age on the occurrence of *O. elegans detorta* (see earlier), and it has been directly dated as Lochkovian on the basis of ostracode biostratigraphy (*325*).

Outgroups of the Actinopterygii + Sarcopterygii clade may provide evidence for a soft maximum age constraint. *Lophosteus superbus*, described on the basis of a wide variety of microremains (*326, 327*) has been considered stem-Osteichthyes (*328*), although this is poorly substantiated (*299, 304, 329*). The earliest occurrence of *L. superbus* from the Pridoli of Gotland (*326, 327*), Estonia (*298*), and Latvia (*298*) is later than the first record of *A. hedei* which, despite concerns over assignment to Actinopterygii (*329*), has not been disputed membership of total-group Osteichthyes. Indeed, some of the evidence on which *Andreolepis* has been assigned to Actinopterygii can be called into question on the basis

of the discovery and phylogenetic position of *Meemania*, in which a ganoine-like tissue appears to be present (*313*). Thus, it is possible that *Andreolepis* presents only osteichthyan ancestral characters and that, on the basis of the available evidence, it is better assigned to stem-Osteichthyes (*304*).

Dating the earliest record of successive sister taxa is complicated by long-standing debate over the relative phylogenetic position and monophyly of the various groups. Acanthodii is generally considered the sister group of Osteichthyes and its earliest record is from the Ashgill of Siberia (*330*). Chondrichthyes is generally accepted as the succeeding sister taxon, the oldest record for which is Caradoc (*60*), although precious few characters bind these remains to the stem of Chondrichthyes (*331*). The oldest placoderms are undescribed forms from the Wenlock of China (*329*) and Vietnam (*332*).

Conservative assessments of the age of the earliest remains readily assignable to the actinopterygian and sarcopterygian total groups are in close approximation (421.75 Ma ± 0 myr vs. 418.7 Ma ± 2.6 myr, respectively). However, phylogenetic assignment of these microremains rests on one or two equivocal derived characters, and this is insufficient evidence on which to justify constraining molecular clock analyses. Thus, we argue that it is best to rely on the evidence of better known and better phylogenetically constrained *Psarolepis* to provide a minimum constraint on the divergence of sarcopterygian and actinopterygian lineages. The firmest age dating on the earliest record of *Psarolepis* (based on biostratigraphic correlation) is 418.7 Ma ± 2.7 myr. Thus, a minimum constraint on the divergence of crown Osteichthyes should be quoted as 416.0 Ma. A soft maximum constraint could be provided by the age of the earliest record of *A. hedei*, dated at 421.75 Ma.

Clupeocephala: zebrafish–Medaka, stickleback, Takifugu, Tetraodon (minimum = 149.85 Ma; soft maximum = 165.2 Ma)

This divergence event represents the splitting of the ostariophysean and euteleost lineages. The earliest ostariophysean is *Tischlingerichthys viohli* from the Tithonian upper Solnholfen Limestone Formation of southern Germany (*333*). It is recognized on the basis of derived characters including the absence of a basisphenoid, and dorsomedial portions of the anterior neural arches expanding and abutting against each other and the posterior margin of the exoccipital.

From the same deposit, Arratia (*333*) also described a number of additional taxa (*Leptolepides,*

Orthogonikleithrus) that qualify as the earliest record of the euteleost lineage. These were assigned to Salmoniformes. The security of their assignments to these higher-level clades within Euteleostei is questionable, although their assignment to the euteleost total-group is not, based not least on the presence of enlarged neural arches/spines.

Thus, earliest representatives of both lineages are in precise agreement. However, this should come as no surprise given that they were found in the same deposit. Therefore, the fossil date is likely to be a considerable underestimate, subject to lagerstätten effect. There are no earlier records.

The dating of the upper Solnhofen Limestone Formation has been based on ammonite zonation and the Formation is assigned to the ti_2 division of the middle Tithonian, Late Jurassic. The Tithonian is dated as 150.8 Ma ± 4.0 myr–145.5 Ma ± 4.0 myr (*240*), but the upper Solnhofen Limestone Formation represents just the middle biohorizon of the lowest ammonite zone of the Tithonian (*334*), its base intercalated by the first (local) appearances of the ammonites *H. hybonotum* (and *Gravesia*) and *Glochiceras lithographicum* (*335*). In proposed stratotype sections, the base of the Tithonian is represented by the simultaneous first appearance of these two taxa plus the immediately subsequent appearance of *Gravesia* species (*240*). The base of the *H. hybonotum* Zone coincides with the base of the normal polarity Chron M22An which is dated at 150.8 Ma ± 0.1 myr (*240*). Given that the Solnhofen Formation falls fully within the *H. hybonotum* Zone, it is possible to derive a lower bound on its age from the base of the succeeding, *S. darwini* ammonite zone which coincides approximately with the M22n Chronozone, dated at 149.9 Ma ± 0.05 myr (*240*).

Thus, the earliest paleontological evidence and, therefore, a lower bound on the split of *Danio rerio–Takifugu rubripes, Tetraodon nigris* can be considered to be 150.8 Ma ± 0.1 myr–149.9 Ma ± 0.05 myr, giving a minimum date of 149.85 Ma. However, note should be taken of the fact that the co-occurrence of the earliest records of these two lineages is an artefact of their presence in a Konservat-lagerstatten. A soft maximum constraint on the divergence of the ostariophysean and euteleost lineages is provided by the census of teleost–total group diversity provided by the assemblages found in the many Oxfordian localities in the Cordillera de Domeyko (*336*). Many species are known in conditions of exceptional preservation and these are stem teleosts; no otophysans or euteleosts are known from here or from older deposits.

The base of the Oxfordian (161.2 Ma ± 4.0 myr; *240*) can be taken as the soft maximum constraint: 165.2 Ma.

Medaka–stickleback, Takifugu, Tetraodon (minimum = 96.9 Ma; soft maximum = 150.9 Ma)
This divergence event represents the split between Atherinomorpha and Percomorpha within Acanthopterygii. The oldest member of Atherinomorpha, based on otoliths of "*Atherinidarum*," from Argile de Gan, Gan, Pyrénées-Atlantiques, France, has been assigned an early Eocene (Ypresian) age (*337*). The earliest skeletal records are late Eocene (Priabonian) (*338*). The oldest percomorph is the stem-tetraodontoform *Plectocretacicus clarae*, from the Cenomanian (Upper Cretaceous) of Hakel, Lebanon (*339, 340*). The age of the lithographic limestones at Hakel is derived from the occurrence of *Mantelliceras mantelli* and the benthic foraminifer *Orbitulina concava* (*341*). The stratigraphic range of *O. concava* is late Albian to early Cenomanian (*342*), while *M. mantelli* defines the basal ammonite zone of the Cenomanian. The base of the *M. mantelli* Zone is well dated on the basis of Ar–Ar and cyclostratigraphy at 99.1 Ma ± 0.4 Ma (*343*). Ogg *et al.* (*344*) provide a date of 97.8 Ma for the top of the *M. mantelli* Zone; errors on the timescale on surrounding zonal boundaries are 0.9 myr. Thus, the minimum age of the divergence of Atherinomorpha and Percomorpha can be based on the minimum age of the lithographic limestones of Hakel, which would be 96.9 Ma.

The most appropriate soft maximum bound on the divergence of Gasterosteiformes and Tetraodontiformes would be the earliest euteleost records, provided by taxa such as *T. viohli* and associated crown euteleosts from the Tithonian of Solnhofen (*333*). Acanthopterygians (as are convincing members of any elopocephalan superorders or orders) are entirely absent. The soft maximum age of the Solnholfen lithographic limestones (justified above in connection with the ostariophysean–euteleost split) is 150.8 Ma ± 0.1 myr. Thus a soft maximum constraint for divergence of the gasterosteiform and tetraodontiform lineages is 150.9 Ma.

Stickleback–Takifugu, Tetraodon (minimum = 96.9 Ma; soft maximum = 150.9 Ma)
This divergence event represents the split between Gasterosteiformes and Tetraodontiformes within Percomorpha. The oldest member of Gasterosteiformes is *Gasterorhamphosus zuppichinii* from the Calcare di Mellissano, near Nardò, Lecce, Apulia, southeastern Italy (*345*), which is believed to be Campanian (Late Cretaceous) in age (*338*). This is younger than the oldest

known member of the tetraodontiform lineage, *P. clarae*, the earliest stem-tetraodontiform, from the Cenomanian (Upper Cretaceous) of Hakel, Lebanon (*339, 340*). The age of the lithographic limestones at Hakel is derived from the occurrence of *M. mantelli* and the benthic foraminifer *O. concava* (*341*). The stratigraphic range of *O. concava* is late Albian to early Cenomanian (*342*), while *M. mantelli* is more restricted temporally, and falls fully within the range of *M. mantelli*, defining the basal ammonite zone of the Cenomanian. The base of the *M. mantelli* Zone is well dated on the basis of Ar–Ar and cycle stratigraphy at 99.1 Ma ± 0.4 Ma (*343*). Ogg *et al.* (*344*) provide a date of 97.8 Ma for the top of the *M. mantelli* Zone; errors on the timescale on surrounding zonal boundaries are 0.9 myr. Thus, the minimum age of the divergence of Atherinomorpha and Percomorpha can be based on the age on the minimum age of the lithographic limestones of Hakel, which would be 96.9 Ma.

Given that *P. clarae* is also the oldest known percomorph (*338*), the most appropriate soft maximum bound on the divergence of Gasterosteiformes and Tetraodontiformes would be the earliest euteleost records, provided by taxa such as *T. viohli* and associated crown euteleosts from the Tithonian of Solnholfen (*333*). Acanthopterygians (as are convincing members of any elopocephalan superorders or orders) are entirely absent. The soft maximum age of the Solnholfen lithographic limestones (justified above in connection with the ostariophysean–euteleost split) is 150.8 Ma ± 0.1 myr. Thus a soft maximum constraint for divergence of the gasterosteiform and tetraodontiform lineages is 150.9 Ma.

Tetraodontidae: Takifugu–Tetraodon (minimum = 32.25 Ma; soft maximum = 56.0 Ma)
Following the phylogenetic scheme of Holcroft (*346*) this divergence event represents the origin of crown-group Tetraodontidae. *Archaeotetraodon winterbottomi* has been identified as a member of this clade on the presence of numerous tetraodontid derived characters, including 11 caudal fin rays, 18 vertebrae, broadened neural and haemal spines and an absence of ribs (*347*). It has been recorded from the Pshekhsky Horizon, in the lower part of the Maikop Formation of the north Caucasus, Russia (*348*), making it the earliest known member of Tetraodontidae (*347*). The lower part of the Maikop Formation has been widely quoted as lower Oligocene (*348, 349*), although evidence is rarely presented in support of this.

Leonov *et al.* (*350*) provide evidence on the age of the Pshekhsky Horizon on the basis of planktic and benthic

foram, nannoplankton and dinocyst biostratigraphy. The base of the Pshekhsky Horizon coincides with the base of the range of *Globigerina tapuriensis*, which belongs to Zone P18 of the Paleogene planktic foram zonation scheme (*351*). The base of P18 equates to the base of the Oligocene, which has been dated at 33.90 Ma ± 0.1 myr (*107*). The top of the Pshekhsky Horizon coincides approximately with the first appearance of the nannoplankton species *Sphenolithus predistentus*, and the base of NP23, a paleogene nannoplankton zone (*350*). The latter has been dated at 32.25 Ma (*107*), the errors on which are negligible, though there will be an inherent uncalculated error on the biostratigraphic correlation to the Caucasus.

Thus, paleontological evidence on the divergence of the lineages leading to *T. rubripes* and *Tetraodon nigroviridis* provides a minimum constraint of 32.25 Ma. Relationships within Tetraodontiformes have been approached from anatomy and molecular phylogenetics, but remain poorly constrained. Nonetheless, the oldest records for the potential sister clades are all of Eocene age and among them, the oldest record is provided by the balistid *Moclaybalistes danekrus*, known from the lower Eocene Mo-Clay (Fur/Ølst) Formation, which has been dated using magnetostratigraphy and biostratigraphy using nannoplankton, dinoflagellate and pollen zones (*352*). The base of the Ølst Formation coincides with base of Dinoflagellate Zone 6 and the base of the *Apectodinium augustum* Zone, which coincides with the base of the Eocene. A soft maximum constraint on the split of *T. rubripes* and *T. nigroviridus* can thus be obtained from the age of the base of the Eocene which has been dated at 55.8 Ma ± 0.2 myr (*107*), thus 56.0 Ma.

Gnathostomata: shark–fish, tetrapod (minimum = 421.75 Ma; soft maximum = 462.5 Ma)
This divergence represents the origin of crown Gnathostomata and the splitting of Chondrichthyes and Osteichthyes.

The oldest possible record of Chondrichthyes is based on isolated scales from the Late Ordovician Harding Sandstone of Colorado (*60*). These scales exhibit a single chondrichthyan derived character, the presence of a neck canal. There is a sequence of younger records, all based on isolated or fragmentary material and attributed to Chondrichthyes on one or, at most, a couple of derived characters (*353–356*). None of these is sufficient to establish the existence of total-group Chondrichthyes, at least to the degree of certainty necessary to calibrate or even constrain a molecular clock analysis. In this

regard, the earliest records that provide adequate evidence of chondrichthyan affinity are of Early to Middle Devonian age: *Doliodus problematicus* from the Emsian of Canada (*357*), and *Pucapampella* from the Emsian of South Africa (*358, 359*). These records are considerably younger than the oldest record of stem-Osteichthyes.

The oldest possible record of Osteichthyes is based on isolated scales, attributed to acanthodians, from the Late Ordovician of Siberia (*330*). Further records of isolated acanthodian scales and spines are known from the Wenlock onwards but, given that the oldest articulated acanthodians are of Devonian age and younger, the degree to which acanthodian-like scales correlate with what is otherwise known of acanthodian anatomy is extremely uncertain.

Thus, the oldest phylogenetically secure record of the divergence of crown gnathostomes is established on the basis of *Andreolepis hedei*, which is at least a stem-Osteichthyan, if not a stem-Actinopterygian (see crown Osteichthyes). The oldest record of *A. hedei* is established on the basis of a graphic correlation composite standard, at 421.75 Ma (see crown Osteichthyes).

A soft maximum constraint can be established on the basis of the oldest phylogenetically secure stem-gnathostome, *Sacabambaspis janvieri*, dated at 462.5 Ma (see crown Vertebrata).

Vertebrata: lamprey–shark, fish, tetrapod
(minimum = 460.6 Ma; soft maximum = 581 Ma)
Establishing a date on this divergence is complicated by debate over the interrelationships of hagfishes, lampreys, and gnathostomes. Hagfishes and lampreys were long united as cyclostomes to the exclusion of gnathostomes (*360*) until in the 1970s a number of authors independently recognized that lampreys and gnathostomes shared a number of morphological characters lacking in hagfishes (*361–363*). Morphology-based cladistic analyses continue to recognize a long and convincing inventory of features supporting this hypothesis of relationships even in the face of universal support for cyclostome monophyly from molecular datasets (*364–366*). In our view, the evidence from molecular data is now so compelling that we accept cyclostome monophyly and the likelihood that many characters hitherto considered derived features of lampreys + gnathostomes are more appropriately interpreted as ancestral vertebrate characters.

Given the above, the divergence of lampreys and gnathostomes equates to the origin of crown vertebrates.

A number of truly ancient fossil vertebrates have been recognized, extending establishment of crown vertebrates

into the Cambrian. These include numerous soft-bodied organisms from the Early Cambrian Chengjiang fauna, including *Yunnanozoon* and *Haikouella*, thought by some to represent early craniates (*367, 368*), and *Zhongjianichthys*, *Myllokunmingia*, and *Haikouichthys*, which exhibit convincing vertebrate derived characters (*369–372*). However, the evidence supporting their crown rather than stem-vertebrate affinity is not sufficiently convincing to justify their use in calibrating or constraining a molecular clock analysis. Similarly, conodonts have been widely touted as crown vertebrates, even stem-gnathostomes (*373*) but, while debate over the affinity of this group continues with its characteristic vigor, it would be inappropriate to use conodonts as evidence for calibrating or constraining the date of divergence of crown vertebrates.

Although there are a number of records of armored stem-gnathostomes from the Early Ordovician (*374–376*), the earliest phylogenetically secure records are *Arandaspis prionotolepis* (*376*) and *S. janvieri* (*377*), the oldest records of which are of Darriwilian age (*375, 378*). The best constraint on these earliest records is provided by Albanesi *et al.* (*378*), who identify co-occurring conodonts as indicative of the *Pygodus anserinus* Zone. Cooper and Sadler (*379*) interpolate a date of 462.2 Ma for the top of this zone; errors on the adjacent boundaries (top of the Darriwilian) are in the order of ±1.6 myr. Thus, this minimum constraint on the divergence of crown vertebrates is 460.6 Ma.

Providing soft maximum bounds on the timing of crown-vertebrate divergence is contentious because of the possibility that some of the Early Cambrian Chengjiang vertebrates can be attributed to the vertebrate crown. Thus, we use as a soft maximum constraint the same evidence we use to constrain the divergence of bilaterian phyla (see crown Bilateria). Thus, a soft maximum constraint on the divergence of the echinoderm and chordate lineages may be taken as 581 Ma.

Olfactores: tunicate–lamprey, shark, fish, tetrapod (minimum = 518.5 Ma; soft maximum = 581 Ma)
This represents the origin of crown Olfactores, the clade comprised of tunicates and vertebrates (*366*).

There are two putative fossil tunicates from the Early Cambrian Chengjiang biota (*380, 381*), though neither is sufficiently convincing to justify its use in calibrating or constraining a molecular clock analysis. Nevertheless, *Zhongjianichthys*, *Myllokunmingia*, and *Haikouichthys* (*369–372*), which are from the same deposit and can be attributed to the vertebrate total group at the very least,

serve as a minimum constraint on the divergence of tunicates and vertebrates.

Thus, the minimum age of divergence of tunicates and vertebrates can be derived from the minimum age of the Yu'anshan Member of the Heilinpu Formation, in which the Chengjiang biota has been found. Unfortunately, this is equivocal because although its local stratigraphic assignment to the *Eoredlichia wutingaspis* Biozone is well constrained and long established (*382*), how this correlates to better-dated sections is not clear, not least because the fauna is largely endemic. The Heilinpu Formation belongs to the Qiongzhu Stage, which is considered to be Atdabanian in age. Thus, a minimum constraint may be provided by the age of the top of the Atdabanian, for which a date of 518.5 Ma is provided in the latest timescale (*383*). It should be noted, however, that this estimate is stratigraphically relatively remote from the nearest geochronological-derived date, and contingent upon the questionable conclusion that the Qiongzhu and Atdabanian are time equivalent.

To provide a soft maximum bound on the timing of the crown Olfactores divergence, we follow the soft maximum bound on divergence of Bilateria (see crown Bilateria). Thus, a soft maximum constraint on the divergence of the echinoderm and chordate lineages may be taken as 581 Ma.

Chordata: cephalochordate–tunicate, lamprey, shark, fish, tetrapod (minimum = 518.5 Ma; soft maximum = 581 Ma)
Given the recent recognition that cephalochordates are the closest relatives to tunicates plus vertebrates, the split between cephalochordates, tunicates, and vertebrates equates to the origin of crown chordates.

There are a number of putative fossil cephalochordates from the Cambrian including *Pikaia graciliens* from the Middle Cambrian Burgess Shale (*384*) and *Cathaymyrus diadexus* from the Early Cambrian Chengjiang fauna (*385*). At best, however, these fossils exhibit only chordate ancestral characters and, therefore, they provide no constraint over the timing of divergence of crown chordates. However, *Zhongjianichthys*, *Myllokunmingia*, and *Haikouichthys* (*369–372*) are from the same deposit and are attributable to the vertebrate total group at the very least.

The minimum age of the Yu'anshan Member of the Heilinpu Formation, from which the Chengjiang biota has been found, is equivocal. This is because, although its local stratigraphic assignment to the *E. wutingaspis* Biozone is well constrained and long established (*382*),

how this correlates to better-dated sections is not clear, not least because the fauna is largely endemic. The Heilinpu Formation belongs to the Qiongzhu Stage, which is considered to be Atdabanian in age. Thus, a minimum constraint may be provided by the age of the top of the Atdabanian, for which a date of 518.5 Ma is provided in the latest timescale (*383*). It should be noted, however that this estimate is stratigraphically, relatively remote from the nearest geochronological-derived date, and contingent upon the questionable conclusion that the Qiongzhu and Atdabanian are time equivalent.

For the soft maximum bound on the timing of crown chordate divergence, we follow the constraints on the divergence of Bilateria (see crown Bilateria). Thus, a soft maximum constraint on the divergence of the echinoderm and chordate lineages may be taken as 581 Ma.

Deuterostomia: sea urchin–cephalochordate, tunicate, lamprey, shark, fish, tetrapod (minimum = 518.5 Ma; soft maximum = 581 Ma)
This divergence event represents the splitting of crown deuterostomes into the chordate and ambulacrarian lineages, the latter clade composed of echinoderms and hemichordates.

The oldest possible record of chordates dates from the Lower Cambrian Yu'anshan Member of the Heilinpu Formation (Chengjiang Biota) of Yunnan Province, South China, from which the remains of putative tunicates (*380, 381*), cephalochordates (*385, 386*), and even vertebrates (*367–372*) have been described. The problem with many of these records is that the characters defining clades at this deep level within phylogeny are largely cytological or embryological—not the kinds of characters that are preserved under even the most exceptional circumstances (*387*). Furthermore, both the living and fossil organisms are entirely soft-bodied and so precious few characters are preserved. And of these, many have been resolved to be deuterostome ancestral characters, rather than chordate or vertebrate-derived characters, with the recognition that echinoderms and hemichordates are sister taxa (*387, 388*). Thus, *Yunnanozoon* and *Haikouella*, thought by some to represent early craniates (*367, 368*), are interpreted by others as basal (perhaps even stem-) deuterostomes (*331, 371, 389–393*). Records of early tunicates (*380, 381*) have been questioned and the earliest unequivocal remains are Triassic in age (*394*). The putative vertebrates *Zhongjianichthys*, *Myllokunmingia*, and *Haikouichthys* (*369–372*) exhibit convincing vertebrate-derived characters, and these provide the best constraint on the minimum date of divergence of vertebrates

and chordates. There are contemporaneous records of more primitive deuterostomes, with the identification of vetulicystids as stem-echinoderms (*389*) and vetulicolians as stem-deuterostomes (*371, 389, 391*), although the veracity of the phylogenetic assignments of these taxa is a matter of some controversy (*395–397*). Earlier records of possible deuterostomes include *Arkarua* from among the enigmatic ediaracan biota (*398*). Although support for the identification of *Arkarua* as an echinoderm has found support from embryological homologies (*399*), all rests ultimately upon the presence of pentameral symmetry, which is not enough to rest an extension of tens of millions of years to a minimum date for divergence of deuterostomes and Bilateria upon. Thus, the vertebrates *Zhongjianichthys*, *Myllokunmingia*, and *Haikouichthys* (*369–372*) provide the best evidence for the minimum date of divergence of deuterostomes.

Thus, a minimum constraint on the divergence of crown deuterostomes is based on the vertebrates *Zhongjianichthys*, *Myllokunmingia*, and *Haikouichthys* and the minimum age of the Yu'anshan Member of the Heilinpu Formation, from which the Chengjiang biota has been found. The age of the Chengjiang biota remains equivocal because, although its local stratigraphic assignment to the *E. wutingaspis* Biozone is well constrained and long established (*382*), how this correlates to better-dated sections is not clear, not least because the fauna is largely endemic. The Heilinpu Formation belongs to the Qiongzhu Stage, which is considered to be Atdabanian in age. Thus, a minimum constraint may be provided by the age of the top of the Atdabanian, for which a 518.5 Ma is provided in the latest timescale (*383*). It should be noted, however that this estimate is stratigraphically, relatively remote from the nearest geochronological-derived date, and contingent upon the questionable conclusion that the Qiongzhu and Atdabanian are time equivalent.

The soft maximum bound on the timing of crown deuterostome divergence is based on the constraints on the divergence of bilaterians (see crown Bilateria). Thus, a soft maximum constraint on the divergence of the echinoderm and chordate lineages may be taken as 581 Ma.

Bilateria/Nephrozoa: arthropod, nematode, annelid, mollusc–echinoderm, chordate (minimum = 531.5 Ma; soft maximum = 581 Ma)
This divergence event represents the splitting of crown Bilateria, and the divergence of deuterostome and protostome lineages.

The are numerous convincing chordates, among other putative deuterostomes, from the Lower Cambrian

Yu'anshan Member of the Heilinpu Formation (Chengjiang Biota) of Yunnan Province, South China (see Deuterostomia, Chordata, Olfactores, and Vertebrata, above), providing a minimum constraint of 518.5 Ma.

The earliest evidence for the origin of arthropods are *Rusophycus*-like trace fossils from the upper Nemakit-Daldynian (early Tommotian) of Mongolia (*400, 401*) (520.5 Ma; see Arthropoda–Nematoda). However, there are still older representatives of the protostome lineage, further constraining the time of divergence of the human and fruitfly genomes, as well as the genomes of all integral taxa. The oldest of these is probably the mollusc *Latouchella* from the middle *Purella* Biozone, Nemakit-Daldynian, of Siberia (*401, 402*). There are a number of candidate crown bilaterians among the Ediacaran biota, among which a molluscan affinity for *Kimberella* has been most cogently argued (*403*). However, the evidence has not withstood scrutiny (*393*) and it is certainly insufficient to justify its use as a calibration for, or constraint on molecular clock analyses of metazoan evolution.

The boundary between the Nemakit-Daldynian and the succeeding Tommotian Stage remains equivocal and so a more reliable minimum constraint might be provided by the current best estimate for the base of the Tommotian, which is 531.5 Ma (*383*). Thus, on the basis of the available paleontological, stratigraphic, and chronological data, the best minimum constraint for the divergence of crown Bilateria is 531.5 Ma.

Providing soft maximum bounds on the timing of crown bilaterian divergence is extremely contentious. Nevertheless, following the same criteria used to provide constraints on other divergence events, it is possible to constrain the timing of divergence of bilaterian phyla on the occurrence of older lagerstätten that preserve records of earlier branching lineages. Inevitably, these records are represented by the Ediacaran faunas, the interpretation of which is extremely contentious, though there is increasing agreement that crown bilaterians are not represented among them (*393, 404*). Thus, the youngest, most completely sampled Ediacaran assemblage may be used to provide the soft maximum constraint on the divergence of bilaterian phyla including the chordate and echinoderm lineages. This is the Doushantuo Formation, which provides a sampling of Ediacaran diversity in a number of facies and through a number of modes of exceptional preservation (*405, 406*); although a number of candidate bilaterians have been described from this deposit (*407–411*), these have not withstood scrutiny (*412–415*). The most exacting test of the existence of bilaterians such as deuterostomes is

provided by the phosphates that occur early within the sequence and include exquisitely preserved embryos and hatchlings of metazoan affinity (*416–418*) and adults of possible cnidarians (*412*). No uncontested bilaterians are present (*417*). Condon *et al.* (*419*) indicate that the phosphorites are younger than the tillites of Gaskiers glaciogenic event which has been dated at 580 Ma ± 1 myr. This date is older than all Ediacarans that have been proposed as bilaterians (*420, 421*). The date provided differs from Benton and Donoghue (*3*) [but not Donoghue and Benton (*4*)], who provided the minimum not the soft maximum age on the embryo-bearing horizons in the Doushantuo Formation.

Protostomia: arthropod, nematode–annelid, mollusk (minimum = 531.5 Ma; soft maximum = 581 Ma)
The constraints on the divergence of Bilateria/Nephrozoa apply as equally to the internal split within Protostomia, between Ecdysozoa and Lophotrochozoa. The principal record providing minimum constraint on the divergence of these lineages is that of the mollusk *Latouchella* (*401, 402*), providing a date of 531.5 Ma (see Bilateria/Nephrozoa). For the soft maximum bound we use evidence presented for the soft maximum bound on divergence of Bilateria (see crown Bilateria). Thus, a soft maximum constraint on the divergence of the ecdysozoan and lophotrochozoan lineages may be taken as 581 Ma.

Annelida–mollusca: leech, polychaete–limpet, sea hare, Biomphalaria (minimum = 531.5 Ma; soft maximum = 581 Ma)
The evidence marshaled to constrain the divergence of Bilateria/Nephrozoa applies equally to the divergence of annelida and mollusca because it is based on the earliest mollusk. The principal record is *Latouchella* (*401, 402*), providing a date of 531.5 Ma (see Bilateria/Nephrozoa); as discussed with regard to Bilateria, we do not consider the evidence supporting a molluscan affinity of the edicaran organism *Kimberella* as sufficient to justify its use as a minimum constraint on the establishment of the molluscan phylum. For the soft maximum bound we evidence presented for the soft maximum bound on divergence of Bilateria (see crown Bilateria). Thus, a soft maximum constraint on the divergence of the annelida–mollusca may be taken as 581 Ma.

Gastropoda: limpet–Biomphalaria, sea hare (minimum = 470.2 Ma; soft maximum = 531.5 Ma)
The divergence of the limpet *Lottia* from the euthyneurans *Biomphalaria* and *Aplysia* represents the divergence

of the two major living subclasses of Gastropoda, Eogastropoda, and Orthogastropoda, respectively, as well as the base of crown Gastropoda. There are many candidates for the earliest crown gastropod but the precise affinity of these early mollusks remains questionable. The earliest secure record of crown Gastropoda may be sought in the oldest vetigastropods, which are conventionally accepted as members of the living gastropod clade. Thus, a minimum constraint would rest on the sinuopeid *Sinuopea sweeti* from the Jordan Sandstone of Wisconsin, and the raphistomatid *Schizopea typical* from the Eminence Dolomite of Missouri (*59*). Both the Eminence Dolomite and Jordan Sandstone are generally quoted to be Trempealeauan in age, but the basis of this age justification is unclear for the Eminence Dolomite (*422*), while the Jordan Sandstone has been assigned to the Sunwaptan on the basis of its trilobite fauna (*423*). Thus, using *S. sweeti*, the minimum constraint on the divergence of crown gastropods would be 490 Ma (*383*). However, Wagner has questioned the reliability with which any early Palaeozoic gastropods may be assigned to each of the three main extant lineages of gastropods, with the exception of the eotomarioids, which he identifies as candidates for the ancestry of extant vetigastropods (*424*). The oldest member of Eotomarioidea is *Turritoma acrea* from the Catoche Formation of Western Newfoundland (*425*). The Catoche Formation falls fully within the *Oepikodus communis* conodont biozone (*425*) the top of which, in the sense that it is employed, coincides with the Ibexian/Whiterockian boundary; this would be the *O. communis* and *Reutterodus andinus* Biozones of Ross and colleagues (*426*). This coincides with the Early/Middle Ordovician Boundary in the 2004 Geologic Timescale, and a date of 471.8 Ma ± 1.6 myr (*379*). Thus, on this conservative view which we follow, the minimum constraint on the divergence of crown Gastropoda is 470.2 Ma.

A soft maximum constraint may be provided by the oldest mollusk, *Latouchella*, dated at 531.5 Ma (see earlier).

Euthyneura: sea hare–Biomphalaria (minimum = 168.6 Ma; soft maximum = 473.4 Ma)
The divergence of the sea hare *Aplysia* and the air-breathing freshwater snail *Biomphalaria* reflects the divergence of Pulmonata and Opisthobranchia, and the base of crown Euthyneura. Molecular phylogenetic analyses indicate that although pulmonates and opisthobranchs are each other's closest relatives, their monophyly is questionable (*427, 428*). On evidence of mitochondrial

synteny, pulmonates have been identified as paraphyletic with respect to a monophyletic Opisthobranchia and among the pulmonates considered, *Biomphalaria* has been identified as more closely related to opisthobranchs than to the other pulmonates included in the analysis (*429*). This indicates that the split between *Biomphalaria* and *Aplysia* does not coincide with the base of crown Euthyneura.

The oldest records of Euthyneura, both opisthobranchs and pulmonates are Tournaisian (*59*) but, because of the uncertainty concerning the interrelationships of Euthyneura it would be safer to rely upon the earliest records of lower rank taxa to which *Biomphalaria* and *Aplysia* have been assigned. The oldest record of the Order Aplysiomorpha to which *Aplysia* has been assigned is Tertiary. The earliest record of Basommatophora, the order to which *Biomphalaria* is assigned, is also Tournaisian, but the monophyly of Basommatophora remains to be established and, thus, a more reliable minimum constraint may instead be provided by the oldest record of the Superfamily Planorboidea and Family Planorbidae to which *Biomphalaria* is assigned. This earliest record is *Anisopsis calculus* from Cajac, France, which is reported to be of Aalenian (Jurassic) age (*59*). Without further constraint, the age of the Aalenian–Bajocian boundary may be used which, following the 2004 Geologic Timescale is 171.6 Ma ± 3.0 myr (*240*). Thus, the minimum constraint on the divergence of *Aplysia* and *Biomphalaria* is 168.6 Ma.

A soft maximum constraint on the divergence of these heterobranch orthogastropods may be provided by evidence for the establishment of Orthogastropoda, dated at 471.8 Ma ± 1.6 myr (see earlier) and, thus, 473.4 Ma.

Capitellid polychaete–leech (minimum = 305.5 Ma; soft maximum = 581 Ma)
The intrarelationships of annelids are in a state of flux, with the phylogenetic signal from competing molecular data sets conflicting with one another, and with morphology-based data sets. Some general conclusions are that clitellates, the clade to which leeches are assigned, are monophyletic, but nest within polychaetes, which are grossly paraphyletic (*430, 431*).

The oldest possible clitellate is a putative leech described from the Middle Silurian of Wisconsin (*432, 433*), and a much younger form from the Jurassic of Solnholfen (*434*). However, the evidence presented in support of their assignment to the clitellates amounts to little more than their vaguely leech-like round mouth and segmented body. *Pronaidites carbonarius* was described as a

Carboniferous oligochaete, but this record requires careful redescription and reconsideration (*435*).

The gross paraphyly of polychaetes renders the significance of Cambrian polychaetes moot; they have been assigned to the extant clade Phyllodocida (*436*) but their assignment to any extant clade within Annelida has recently been challenged (*437*). Polychaetes are well represented by their jaw elements in the fossil record, from the Early Ordovician onward (*438*), but although they are often considered eunicids, there is no real evidence to support this. This is unfortunate because although the precise affinity of eunicids is unclear in recent molecular phylogenies, in one manner or another, eunicids, along with amphinomid and flabelligerid polychaetes, intercalate the clade circumscribed by clitellates and capitellid polychaetes (*430, 431*). Thus, we may derive a minimum constraint from the oldest securely identified fossil eunicid, amphinomid, or flabelligerid, all of which (e.g., *Esconites zelus*, *Rhaphidiophorus hystrix*, and Flabelligeridae sp., respectively) are from the Pennsylvanian Mazon Creek fauna of Illinois (*439, 440*). The Mazon Creek fauna is derived from the Francis Creek Member of the Carbondale Formation in NE Illinois. The Francis Creek Shale Member has been dated as middle Desmoinesian and middle Westphalian D age on the basis of both palynological and paleobotanical data (*441–443*). This equates to the upper part of the Moscovian Stage, the top of which has been dated at 306.5 Ma ± 1.0 myr on the basis of a graphically correlated composite standard calibrated using radiometric dates (*252*). The top of the Westphalian D is slightly older at 307.2 Ma. (*252*). Thus, the minimum constraint on the divergence of *Capitella* from *Helobdella* is 305.5 Ma.

To provide a soft maximum bound on the timing of capitellid polychaete–leech divergence, we follow the soft maximum bound on divergence of Bilateria (see crown Bilateria). Thus, a soft maximum constraint on the divergence of the capitellid polychaete and clitellate lineages may be taken as 581 Ma.

Nematode–arthropod (minimum = 520.5 Ma; soft maximum = 581 Ma)

This divergence event represents the splitting of the nematode and arthropod lineages.

The earliest evidence for the origin of arthropods are *Rusophycus*-like trace fossils from the upper Nemakit-Daldynian (early Tommotian) of Mongolia (*400, 401*). Dating of the Early Cambrian is not well advanced, not least because a global scheme of stratigraphic zonation for the Early Cambrian has yet to be established.

The most appropriate date for constraining the age of these trace fossils is the top of the Tommotian, the Tommotian–Atdabanian boundary. The best available date for this is 522.5 Ma, provided by Shergold and Cooper (*383*), though it is an estimate based on younger and older geochronological dates, errors on which are reported in the order of 2 myr. Thus, the minimum constraint on the divergence of arthropod and nematode lineages is 520.5 Ma.

There are older representatives of mollusks, and we may consider these in establishing soft maximum bounds on the timing of arthropod–nematode divergence. The oldest mollusk is *Latouchella*, from the middle *Purella* Biozone, Nemakit-Daldynian, of Siberia (*401, 402*). This indicates the existence of the ecdysozoan total group to which nematodes and arthropods belong, but this isolated record provides no confidence on which to judge whether or not the arthropods and nematode lineages had yet diverged. There are a number of candidate crown bilaterians among the Ediacaran biota, among which a molluscan affinity for *Kimberella* has been most cogently argued (*403*). However, the evidence has not withstood scrutiny (*393*) and it is certainly insufficient to justify its use as a constraint on molecular clock analyses of metazoan evolution. Thus, we follow the soft maximum constraint on the date of divergence of crown Bilateria, 581 Ma.

Mandibulata: Daphnia–louse, Rhodnius, aphid, beetle, wasp, honeybee, mosquito, fruitfly (minimum = 510 Ma; soft maximum = 543 Ma)

This represents the establishment of crown Mandibulata and the divergence of Crustaceomorpha from Atelocerata. The fossil record of crustaceans is by far the more extensive of the two lineages comprising Atelocerata. The earliest possible crustaceans have been reported from the Mount Cap Formation of northwestern Canada (*444*). However, these remains are fragmentary and their interpretation as crustaceans is based on the special similarity between individual fragments and the filter-feeding apparatus of modern branchiopod crustaceans, rather than on the basis of a suite of mutually corroborative phylogenetically informative characters.

The earliest convincing evidence for the divergence of Atelocerata and Crustaceamorpha is the phosphatocopid *Klausmuelleria salopiensis* from the Lower Cambrian Comley Limestones of Shropshire, UK (*445–447*). Siveter *et al.* (*447*) indicate that the lower Comley Limestones can be assigned to the Protolenid-Strenuellid Biozone which correlates to the Botomian-Toyonian age within the

Siberian stratigraphic framework (*447*). Within the 2004 Geologic Timescale, this provides a minimum constraint of 510 Ma (*383*).

A soft maximum constraint may be provided by the earliest evidence of arthropods, based upon *Rusophycus*-like trace fossils (see "Nematoda–Arthropoda" later) from the Nemakit-Daldynian (early Tommotian) of Mongolia (*400, 401*). A soft maximum constraint may therefore be derived from the base of the Nemakit-Daldynian which equates to the base of the Cambrian and, thus, 542 Ma ± 1.0 myr (*383*). Our soft maximum constraint is therefore 543 Ma.

Eumetabola: louse, Rhodnius, aphid–beetle, wasp, honeybee, mosquito, fruitfly (minimum = 307.2 Ma; soft maximum = 414 Ma)

The divergence of Paraneoptera from Holometabola. Providing a minimum constraint on the divergence of crown Eumetabola is complicated by the lack of resolution concerning the affinity of Palaeodictyopterida, which has been variably considered a member of the clade. Grimaldi and Engel exclude palaeodictyopterids from the clade, leaving Miomoptera as the oldest members of Eumetabola (*448*). These authors discuss the various possible affinities of Mimptera among Paraneoptera or Holometabola, but there appears no equivocation of their membership of Eumetabola. The oldest known record of Miomoptera is an undescribed specimen (Field Museum PE 293590 from the Pennsylvanian Mazon Creek Lagerstatte (*449*)). The Mazon Creek fauna is derived from the Francis Creek Member of the Carbondale Formation in NE Illinois. The Francis Creek Shale Member has been dated as middle Desmoinesian and middle Westphalian D age on the basis of both palynological and paleobotanical data (*441–443*). This equates to the upper part of the Moscovian Stage, the top of which has been dated at 306.5 Ma ± 1.0 myr on the basis of a graphically correlated composite standard calibrated using radiometric dates (*252*). The top of the Westphalian D is slightly older at 307.2 Ma. (*252*). Thus, the minimum constraint on the divergence of crown Eumetabola is 305.5 Ma.

A soft maximum constraint may be provided by the age of the oldest insect *Rhyniognatha hirtsi* from the Early Devonian Rhynie Chert of northeast Scotland (*450*). The age of the Rhynie Chert has been best established on the basis of the composition of its spore assemblages which indicate an early Pragian to the earliest Emsian age span (*451*). Thus, we may establish a soft maximum constraint on the base of the Pragian which is 411.2 Ma ± 2.8 myr (*452*), equating to 414 Ma.

Paraneoptera: louse–Rhodnius, aphid (minimum = 283.7 Ma; soft maximum = 414 Ma)

Though the assignment of Archescytinidae to the hemipteran crown group may be questioned, there is no question of its membership of Paraneoptera. There are older records of Paraneoptera, including Permopsocidae, but these are likely stem-Paraneoptera (*25, 448*). Thus, the best minimum constraint on the divergence of Paraneoptera is provided by an undescribed archescytinid from the middle Bacov Beds of Boscovice Furrow, Obora Czech Republic (*453, 454*). These rocks were described as Artinskian by Kukalová-Peck and Willmann (*454*), without justification, but they have subsequently been attributed to the Sakmarian using vertebrate microremains for biostratigraphic correlation (*455, 456*). On this basis we may use the top of the Sakmarian as our basis for a minimum constraint on the divergence of Paraneoptera and Holometabola which is as given as 284.4 ± 0.7 myr (*457*), providing the minimum constraint of 283.7 Ma.

The most approximate soft maximum constraint on the divergence of Paraneoptera is provided by the earliest records of Neoptera, which are a paraphyletic assemblage of Late Carboniferous roach-like dictyopterans, sometimes grouped as the grade Blattodea or Blatoptera. The oldest such record is probably *Ctenopilus elongatus* (previously *Eoblattina complexa*) from the Stephanian B-C of the Commentary Basin, France (*458*). The Stephanian B of western Europe correlates to the upper Kasimovian of the 2004 Geologic Timescale, the base of which has been dated at 306.5 Ma ± 1.0 myr (*252*) and, thus, a soft maximum constraint of 307.5 Ma. However, given the reliance on temporally isolated lagerstatten for constraining the temporal diversification of insects, this envelope is perhaps too strict. Instead, a more appropriate soft maximum constraint may be provided by the oldest member of Pterygota, the oldest possible record of which is also the oldest known insect, *Rhyniognatha hirsti* (*450*), providing a constraint of 414 Ma (see Eumetabola earlier).

Hemiptera: Rhodnius–aphids (minimum = 199.0 Ma; soft maximum = 307.5 Ma)

The oldest known hemipterans are members of the Archescytinidae, the oldest record of which remains undescribed but has been recorded from the early Artinskian locality of Obora (*453*). Archescytinidae is identified by Shcherbakov and Popov as more closely related to aphids than to Cimicina and, hence, providing a minimum constraint on the split between *Rhodnius* and aphids (*453*). However, Engel and Grimaldi question this interpretation of the affinity of Archescytinidae

within Hemiptera because the necessary characters are not preserved. Engel and Grimaldi (p. 321) describe three unnamed heteropterans from the Triassic of Virginia (USA), but the oldest described taxon is the *Lufengnacta* (Corixidae, Nepomorpha, Panheteroptera, Heteroptera) from the Yipinglang Coal Series of Yunnan Province, China. The age of the Yipinglang Coal Series is widely agreed to be of Late Triassic age and has been used to justify the correlation of overlying units across South China. Its precise age may be constrained by the palyno-flora (*459*) which provides a Rhaetian-Norian age. Thus, the minimum constraint on the divergence of crown Hemiptera is provided by the date for the end Rhaetian (end Triassic), which is 199.6 Ma ± 0.6 myr (*267*) and, thus, 199.0 Ma.

A suitable soft maximum constraint may be provided by the earliest Neopteran, which is *C. elongatus* (*458*), providing a date of 307.5 Ma (see Paraneoptera earlier).

Holometabola: beetle–wasp, honeybee, mosquito, fruitfly (minimum = 307.2 Ma; soft maximum = 414 Ma)
Divergence of Coleoptera and Hymenoptera–Panorpida, and the establishment of crown Holometabola. The oldest recorded member of this clade appears to be an undescribed member of Coleopteroidea from the middle Carboniferous Mazon Creek fauna of Illinois, USA (*449*). The Mazon Creek fauna is derived from the Francis Creek Member of the Carbondale Formation in NE Illinois. The Francis Creek Shale Member has been dated as middle Desmoinesian and middle Westphalian D age on the basis of both palynological and paleobotanical data (*441–443*). This equates to the upper part of the Moscovian Stage, the top of which has been dated at 306.5 Ma ± 1.0 myr on the basis of a graphically correlated composite standard calibrated using radiometric dates (*252*). The top of the Westphalian D is slightly older at 307.2 Ma. (*252*) Thus, the minimum date on the divergence of these two clades is 307.2 Ma.

A suitable soft maximum constraint may be provided by the earliest member of Pterygota, which is *R. hirsti* (*450*), providing a constraint of 414 Ma (see Eumetabola earlier).

Hymenoptera–Panorpida: wasp, honeybee–fruitfly, mosquito (minimum = 238.5 Ma; soft maximum = 307.2 Ma)
This divergence event represents the splitting of the Hymenoptera and Panorpoidea lineages.

The earliest recognized Panorpoidea are the mecopteroids that are interpreted as stem-Panorpoidea (or panorpoideans) and are known from records as early as the Permian, the very oldest of which are members of Kaltanidae, interpreted as stem-panorpoideans (*460*).

The earliest recognized Hymenoptera are from the Middle Triassic of Central Asia (*461, 462*), and the Upper Triassic of Australia (*463*) and Africa (*464*), all of which are referred to the Archexyelinae within Xyelidae. This difference in first records of Hymenoptera and Panorpoidea has led to the suggestion that putative stem-panorpoideans from the Permian are unified on shared ancestral characters of Panorpoidea + Hymenoptera (*448*). Thus, the minimum date for the divergence of Hymenoptera and Panorpoidea would be based on the earliest records from the Middle Triassic Madygen Formation of Central Asia (*461, 462*), which is dated as Ladinian and/or Carnian on the basis of palynological data (*465, 466*). In the absence of greater biostratigraphic control it is possible only to derive a minimum date from the base of the Norian (base Norian 216.5 Ma ± 2.0 myr; *267*). Thus, a minimum constraining date would be 214.5 Ma.

However, this inconsistency is predicated upon the assumption that Hymenoptera and Panorpoidea are sister taxa, a view that is not universally accepted. Rasnitsyn (*467*), for instance, maintains that Hymenoptera and Panorpoidea are more remotely related, the closest relatives of Panorpoidea being Neuropteroidea and Coleopteroidea (united on modified ovipositor (gonapophyses 9 (= dorsal valvula) lost, and the intromittant function transferred to gonocoxa 9 + gonostylus 9 (= valvula 3). In this view, Panorpoidea + Neuropteroidea + Coleopteroidea diverged from the lineage leading to Hymenoptera within the paraphyletic Order Palaeomanteida, at a time approximating to the Carboniferous/Permian boundary. Unfortunately, the systematics of this group are poorly resolved and it is unclear which represent the earliest members of the lineages ultimately leading to Panorpiodea and Hymenoptera. The best estimate must be provided by the earliest member of the clade Panorpoidea + Neuropteroidea + Coleopteroidea, but note should be taken of the fact that this date is likely to be extended in light of systematic revision of Palaeomanteida. The oldest known member of Coleoptera is *Pseudomerope gallei*, from the Asselian (299–294.6 Ma ± 0.8 myr) (Lower Permian) of Rícany, Czech Republic (*454*), though the basis of this age assignment is not clear.

The oldest recorded member of this clade appears to be an undescribed member of Coleopteroidea from the middle Carboniferous Mazon Creek fauna of Illinois,

USA (*449*), providing a date of 307.2 Ma (see Eumetabola, earlier). Thus, within the phylogenetic milieu which posits that Hymenoptera are not immediate sister taxa (*467*), the minimum date on the divergence of these two clades is 307.2 Ma.

In conclusion, however, it must be emphasized that Hymenoptera and Panorpoidea are conventionally viewed as sister taxa. Nevertheless, a minimum date for divergence of 214.5 Ma postdates the minimum date of is 238.5 Ma for the divergence of the lineages leading to *Drosophila melanogaster* and *Anopheles gambiae*. *Apis mellifera* falls outside this clade and so in the absence of better constraint over the interrelationships of Diptera and Hymenoptera, a minimum date for their divergence can be taken as 238.5 Ma. A soft maximum constraint can be provided by the less likely hypothesis that Panorpoidea are more closely related to Neuropteroidea and Coleopteroidea, using the oldest record of this clade, described earlier as 307.2 Ma.

Apocrita: honeybee–wasp (minimum = 152 Ma; soft maximum = 243 Ma)
The divergence of the honeybee *Apis* from the parasitic wasp *Nasonia* corresponds to the crown-group concept of the hymenopteran suborder Apocrita, and the divergence of Proctotrupoidea and Chalcidoidea, respectively. The oldest records of both lineages are at minimum, Late Jurassic in age, but the earliest records of Proctotrupoidea are the best dated. These records belong to Mesoserphidae, such as *Mesoserphus* and *Karatoserphus*, from the Early Jurassic Daohugou Beds of Inner Mongolia, China (*468, 469*). The age of these beds has been constrained radiometrically using U-Pb series dating to the interval 168–152 Ma (*470, 471*) and, thus, we take 152 Ma as the minimum constraint on the divergence of honeybee and wasp.

A soft maximum constraint can be provided by the earliest record of Hymentoptera, the earliest recognized members of which are from the Middle Triassic Madygen Formation of Central Asia (*461, 462*), that is dated as Ladinian and/or Carnian on the basis of palynological data (*465, 466*). Thus, the constraint may be derived from the base of the Ladinian, which may be as much as 241 Ma ± 2.0 myr (*267*), equating to a soft maximum constraint of 243 Ma.

Diptera: fruitfly–mosquito (minimum = 238.5 Ma; soft maximum = 295.4 Ma)
This divergence event represents the splitting of Brachycera and Culicomorpha lineages. The oldest representative

of Culicomorpha is *Aenne triassica* from the Late Triassic (Rhaetic) Cotham Member of the Lilstock Formation, Penarth Group at Aust Cliff, near Bristol, UK (*472*). Although this displays chironomid derived characters, only the distal half of a wing is preserved. The base of the Cotham Member coincides with the base of SA5n.3r which equates to the E23r reverse polarity magnetozone of the Newark Supergroup (*473*), the base of which is estimated at 202 Ma ± 1 myr on the basis of volcanics in the upper part of the underlying E23 normal polarity magnetozone (*267*). Hounslow *et al.* (*473*) argue that the whole of the Cotham Member equates to the E23r magnetozone, the duration of which is beyond stratigraphic resolution in the current timescale (*267*). Thus, we conclude the age of the first possible representative of Culicomorpha to be 202 Ma ± 1 myr.

The next oldest record is *Aenne liasina* from the lower Toarcian (Lower Jurassic) of Grimmen, NE Germany (*474*), followed by an abundance of other Culicomorpha records in the Lower and Middle Jurassic (*448*).

The oldest documented representatives of Brachycera are from the Upper Triassic Dan River Group of Virginia (*475, 476*), although their assignment rests upon precious few and largely inconsistent venation characters (*448*). There remains an older record of Brachycera, *Gallia alsatica*, from the Grès-à-Voltzia Formation of Arzviller, northeast France (recognized on the basis of the following derived characters: cell *m3* narrowed distally and Cu and A_1 terminating in one point at the wing margin) (*476, 477*). The Grès à Meules facies of the Grès-a-Voltzia Formation, from which these remains are derived, has been dated as lower Anisian (*478, 479*), although the evidence on which this is based was not presented. The top of the lower Anisian is dated as 240.5 Ma, based on proportional scaling of major conodont zones (*267*) from a graphic correlation global composite standard (*480*), from which an error of ±2.0 myr is derived. Otherwise, there are convincing records from the Early Jurassic, including the Black Ven Marls (Sinemurian) at the cliff of Stonebarrow Hill near Charmouth, Dorest, UK (*turneri-obtusum* Zone) 194.1–192.0 Ma (*481*), and the lower Toarcian (*Harpoceras falciferum* Zone) of Dobbertin, Mecklenburg, Germany 182.7–181.2 Ma (*482*).

The oldest representatives of the clade comprising Culicomorpha and Brachycera are members of grauvogeliid Psychodomorpha, specifically, *Grauvogelia arzvilleriana* from the Middle Triassic Grès-a-Voltzia Formation of France (*483*). Crucially, this is neither the most primitive crown dipteran, nor the oldest known total-group

dipteran, but the oldest record that falls within the clade circumscribed by *Anopheles* and *Drosophila*, following the phylogenetic scheme presented in (*448*).

Thus, on the record of *G. arzvilleriana* (*483*), its coincidence with the earliest (albeit undocumented) record of Brachycera (*476, 477*), and the phylogenetic hypothesis of Grimaldi and Engel (*448*), the minimum date for the divergence of the lineages leading to *D. melanogaster* and *A. gambiae* is 238.5 Ma.

A soft maximum constraint is provided by the insect fauna of Boskovice Furrow, Oboro, Moravia, Czech Republic. A huge diversity of insects has been described from this deposit which is the single most important Paleozoic insect locality in the world (*448*). No members of the clade circumscribed by Brachycera and Culicomorpha have been described from here or from older deposits. The Oboro fauna has been dated at early Artinskian (*454*) and Sakmarian (*456*), although only the latter has been substantiated. The base of the Sakmarian has been dated at 294.6 Ma ± 0.8 myr (*457*). Thus, the soft maximum constraint on the divergence of Brachycera and Culicomorpha can be taken as 295.4 Ma.

Eumetazoa: Cnidaria–Bilateria (minimum = 531.5 Ma; soft maximum = 581 Ma)

The split between Cnidaria and Bilateria represents the origin of crown Eumetazoa. The oldest unequivocal record of Bilateria is the mollusc *Latouchella* from the middle *Purella* Biozone, Nemakit-Daldynian, of Siberia (*401, 402*). In the absence of better constraint, a numerical date may be derived from the boundary between the Nemakit-Daldynian and the succeeding Tommotian Stage. However, this remains equivocal and so a more reliable minimum constraint might be provided by the current best estimate for the base of the Tommotian, which is 531.5 Ma (*383*). Thus, on the basis of the available paleontological, stratigraphic, and chronological data, the oldest record of Bilateria is 531.5 Ma.

The oldest possible record of a cnidarian is provided by *Sinocylcocyclicus guizhouensis* from the Ediacaran Doushantuo phosphorites (*412*), although the evidence in favor of a cnidarian affinity does not amount to more than its structural resemblance to tabulate corals. Innumerable putative medusoid cnidarians have been described among the Ediacaran biota, but these have been reinterpreted as microbial communities (*484*) or trace fossils (*485*). Frond-like Ediacarans such as *Charnia*, have traditionally been interpreted as sea pens, but this comparison is unconvincing (*486, 487*). *Namapoikia rietoogensis* is a slightly younger Ediacaran

record from the Nama Group of northern Namibia (*488, 489*) and, although it exhibits colonial organization and, therefore has drawn comparison to cnidarians, its affinities are speculative nevertheless. *Namacalathus hermanastes* is also known from the Nama Group of central and southern Namibia (*490*); it has also drawn comparison to cnidarians and this comparison is equally equivocal.

Less equivocal records of cnidarians are to be found among Tommotian-age small shelly faunas, represented by anabaritids (*491*) and, later, the tentaculitids. The affinity of both has been the subject of debate but, on the basis of the available evidence, their assignment to the cnidarian total group is compelling. An almost complete life series from embryo to adult of the putative scyphozoan cnidarian *Olivooides* is known to co-occur with elements of the small shelly fauna (*492, 493*), although its affinities are equivocal. The earliest phylogenetically secure cnidarians are corals, based on slightly older Early Cambrian records from North America, Australia, and Siberia (*494, 495*). The oldest coral is probably *Cysticyathus tunicatus* from the Tommotian of Siberia (*496*) and, thus, a numerical constraint on the oldest secure record of a cnidarian can be derived from the age of the top of the Tommotian, the Tommotian–Atdabanian boundary. The best available date for this is 522.5 Ma, provided by Shergold and Cooper (*383*), though it is an estimate based on younger and older geochronological dates, errors on which are reported in the order of 2 myr. This date is significantly younger than the oldest bilaterian record which, at 531.5 Ma, we adopt as the minimum constraint on the divergence of Eumetazoa.

A soft maximum bound could be codified on the basis of the more equivocal cnidarian records outlined earlier. All of these, including the Ediacaran records, are younger than the soft maximum bound established for Bilateria (581 Ma), which also encompasses the Doushantuo record of *S. guizhouensis* (*412*). Thus, we adopt the maximum date on the embryo-bearing horizons in the Doushantuo Formation as our soft maximum constraint on the divergence of crown Eumetazoa, at 581 Ma.

Cnidaria: Hydra–sea anemone (minimum = 520.5 Ma; soft maximum = 581 Ma)

This divergence represents the divergence of Anthozoa (sea anemones—including *Nematostella*, and corals) and Medusozoa (Scyphozoa, Cubozoa, Hydrozoa—including *Hydra* and Staurozoa) and the establishment of crown Cnidaria (*497*). As discussed earlier in connection with Eumetazoa, the oldest records of crown cnidarians are represented by anabaritids (*491*) and the putative

scyphozoan cnidarian *Olivooides* (*492, 493*), although its affinities are equivocal. However, the earliest phylogenetically secure cnidarians are meduzoans from the Middle Cambrian of Utah (*498*) and anthozoans (corals) from the Early Cambrian of North America, Australia, and Siberia (*494, 495*). The oldest coral is probably *C. tunicatus* from the Tommotian of Siberia (*496*) and, thus, a numerical constraint on the oldest secure record of a cnidarian can be derived from the age of the top of the Tommotian, the Tommotian–Atdabanian boundary. The best available date for this is 522.5 Ma, provided by Shergold and Cooper (*383*), though it is an estimate based on younger and older geochronological dates, errors on which are reported in the order of 2 myr, yielding a minimum constraint of 520.5 Ma.

A soft maximum bound could be codified on the basis of the more equivocal cnidarian records outlined earlier (see Eumetazoa). All of these, including the Ediacaran records, are younger than the soft maximum bound established for Bilateria (581 Ma), which also encompasses the Doushantuo record of *S. guizhouensis* (*412*). Thus, we adopt the maximum date on the embryobearing horizons in the Doushantuo Formation as our soft maximum constraint on the divergence of crown Cnidaria, at 581 Ma.

Metazoa: Porifera–Eumetazoa (minimum = 634.97 Ma; soft maximum = 836 Ma)

This divergence event coincides with the origin of crown Metazoa. Dating the divergence of sponges from the lineage leading to cnidarians and bilaterians is complicated by molecular phylogenies which, in contrast to morphology-based analyses (*499, 500*), have resolved Porifera as paraphyletic, composed of as many as three distinct clades of phylum status, with the homoscleromorphs, calcisponges, and demosponges as successive sister taxa to Eumetazoa (*91, 501–505*). In what follows, we specifically aim to constrain the date of divergence of demosponges from the lineage leading to calcisponges, homoscleromorphs, and eumetazoans (cnidarians, aceols, and triploblast bilaterians). This is because our focus is to constrain the divergence of *Renieria*, a demosponge which has been targeted for genome sequencing.

Many of the more ancient and speculative records of cnidarians have also been attributed to the sponges, including *Namacalathus* (*488*) and *Namapoikia* (*490*), but none is entirely convincing. This includes putative sponges from the Ediacaran Doushantuo Formation (*506*), where the structures interpreted as evidence of poriferan affinity are just as readily interpreted as fabric of diagenetic mineralization (*414*). Other problematica that have been attributed to Porifera include the archaeocyaths (*507*), stromatoporoids (*495*), and chancelloriids (*508, 509*), but their earliest records are younger than *Paleophragmodictya* (*510*), which is from the Ediacaran of southern Australia and, as such, it is the oldest convincing record of a sponge (*495*). Its precise taxonomic affiliation is of little consequence so long as it falls within the clade circumscribed by demosponges and all other metazoans which, as a hexactinellid, it does.

All of these records are, however, eclipsed by a biochemical record of demosponges, the precise dating of which is unclear, but which extends between sedimentary deposits representative of the Sturtian and Marinoan glaciations (*511*). On this basis, the age of the Marinoan glaciation can provide a minimum constraint on the divergence of demosponges from other metazoans, dated at 635.51 Ma ± 0.54 myr (*419*); as dating improves for the Oman sequence from which the biomarker record occurs, this date will be revised upward by tens of millions of years.

A soft maximum constraint can be provided by Neoproterozoic lagerstatten, such as the Bitter Springs Formation of central Australia (*512*) and the Svanbergfjellet Formation of Spitsbergen (*513*), that exhibit cellular-level preservation of a diversity of organisms including prokaryotes, sphearomorphic acritarchs, multicellular algae, and various problematica, but no evidence of metazoans, or anything that could even be interpreted as a stem-metazoan. The Bitter Springs and Svanbergfjellet floras have been determined to be of comparable middle Neoproterozoic age on the basis of a global carbon isotope excursion (*514, 515*). There is no direct dating on either formation but the Bitter Springs Formation has been correlated with the volcanic sequence in the upper Loves Creek Formation which has itself been allied with the Gairdner Dyke Swarm (*516, 517*), dated at 827 Ma ± 6 Ma (*518*). Halverson *et al.* (*515*) argue for a younger date, but this is not well substantiated. Thus, we take 836 Ma as the soft maximum constraint on the divergence of the crown Metazoa.

Conclusions

We are on the verge of a new age of dating the tree of life. The decades up to now have been characterized by many improvements in methods and assumptions, but also by tension and squabbling between paleontologists and molecular clock practitioners, and within the

paleontological and molecular camps. We have suggested that a certain amount of that squabbling has been unhelpful or misguided, because people were to some extent talking past each other.

We identify a number of major advances in the last years. Paleontologists are beginning to explore the quality of their data and they are learning to provide the information that is required by molecular analysts. A clearer understanding of how fossils and dates relate to phylogenetic trees, and greater clarity about stem grades and crown clades, have sharpened the debate. The vision of a molecular clock with minimal, especially paleontological, assumptions is giving way to a view that realistic dates for evolutionary events can only be obtained by integrating a greater number of less-constraining assumptions, particularly concerning the nature of fossil distribution and the nature of the rock record. Further, new insights and new algorithms are providing better tools for tree analysis that take account of the reality of the uncertainty in paleontological data.

More work is needed by paleontologists and geologists to clarify specific dates, and to tighten their precision further. In addition, paleontologists must become less optimistic in their claims about "the oldest X," even though this might mean fewer papers in *Science* and *Nature*. On the molecular side, intense study is needed to identify genes, and classes of genes, that are phylogenetically informative and phylogenetically uninformative. Of course, more sequences are needed, especially for previously unsequenced minor classes and phyla—such minor taxa, often "living fossils," can be crucial in pinpointing the origins of major clades.

Dating the tree of life is a grand enterprise, and it is a privilege to live through such times of major change and discovery.

Acknowledgments

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