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Journal

Proceedings of the Royal Society B, 284(1854)

ISSN

0962-8452

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Publication Date

2017-05-17

DOI

10.1098/rspb.2017.0241

Peer reviewed



Research

Cite this article: Motani R, Jiang D-yong, Tintori A, Ji C, Huang J-dong. 2017 Pre- versus post-mass extinction divergence of Mesozoic marine reptiles dictated by time-scale dependence of evolutionary rates. *Proc. R. Soc. B* **284**: 20170241.
<http://dx.doi.org/10.1098/rspb.2017.0241>

Received: 6 February 2017
 Accepted: 24 April 2017

Subject Category:
 Palaeobiology

Subject Areas:
 palaeontology, evolution

Keywords:
 Bayesian morphological clock, divergence time, time-scale dependence, Mesozoic marine reptile, end-Permian mass extinction

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Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3768821>.

Pre- versus post-mass extinction divergence of Mesozoic marine reptiles dictated by time-scale dependence of evolutionary rates

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The fossil record of a major clade often starts after a mass extinction even though evolutionary rates, molecular or morphological, suggest its pre-extinction emergence (e.g. squamates, placentals and teleosts). The discrepancy is larger for older clades, and the presence of a time-scale-dependent methodological bias has been suggested, yet it has been difficult to avoid the bias using Bayesian phylogenetic methods. This paradox raises the question of whether ecological vacancies, such as those after mass extinctions, prompt the radiations. We addressed this problem by using a unique temporal characteristic of the morphological data and a high-resolution stratigraphic record, for the oldest clade of Mesozoic marine reptiles, Ichthyosauromorpha. The evolutionary rate was fastest during the first few million years of ichthyosauromorph evolution and became progressively slower over time, eventually becoming six times slower. Using the later slower rates, estimates of divergence time become excessively older. The fast, initial rate suggests the emergence of ichthyosauromorphs after the end-Permian mass extinction, matching an independent result from high-resolution stratigraphic confidence intervals. These reptiles probably invaded the sea as a new ecosystem was formed after the end-Permian mass extinction. Lack of information on early evolution biased Bayesian clock rates.

1. Introduction

There is systematic discrepancy between the oldest fossil record of a clade and the clock-based estimate of its divergence time. The primary reason for this discrepancy is uncontroversial—the fossil record is incomplete, so the oldest fossil of a clade does not provide its exact divergence time but only the youngest possible estimate for it [1]. Recent studies have revealed that this tendency is independent of the data type, i.e. the use of morphological clocks led to similar but even more exaggerated discrepancies between fossil- and clock-based estimates compared with the molecular clock [2,3]. However, some have suggested that the discrepancy is unrealistically large in some cases to be explained simply by the incompleteness of the fossil record. For example, clock-based estimates for placental mammals have been claimed to be too old, even given the incompleteness of the fossil record, because a birth–death model suggests that such a deep divergence time would require much slower diversification rates than

what are preserved in fossils [4], and minor changes in the clock rate could lead to overestimation of divergence time [2]. Similarly, large gaps between two realms of estimates exist in squamates [5,6] and teleosts [7,8], with one or more mass extinction event(s) sandwiched between the oldest fossil record and rate-based divergence time.

Continuous efforts have been made in Bayesian phylogenetics to improve the accuracy of molecular rate estimation and divergence time dating through incorporation of increasingly realistic models of molecular evolution, e.g. introduction of tip dating [9–11], refinements of clock models [12,13] and incorporation of fossilized birth–death processes [14]. A continuing controversy regarding rate estimation is the time-scale dependence of evolutionary rates: estimated evolutionary rates have been shown to correlate negatively with divergence time, especially when tips rather than the nodes are used for time constraints [15–18]. Multiple factors probably underlie this correlation, such as loss of the initial genetic variations through purifying selection of weakly deleterious mutations [16] and the lack of data on ancestral polymorphism [19]. Time-scale dependence of evolutionary rates has been known in morphological studies for over three decades [20–22], although mostly without a phylogenetic framework. More recently, the Bayesian phylogenetic framework allowed the use of morphological clocks to estimate evolutionary rates, opening new possibilities for testing the dependence. Early studies of Bayesian morphological clocks showed that divergence time estimates from morphological rates resulted in even greater discrepancy with the fossil record than with molecular rates [2,3]. However, the time-scale dependence of morphological rates has yet to be specifically addressed under this framework.

Palaeontological studies have established that apparent evolutionary rates, quantified as rates of diversification [23] or morphospace occupancy [24], increased after mass extinctions [25]. If the aftermath of mass extinction also affects morphological or molecular clock rates in a Bayesian phylogenetic sense, then mass extinction may affect the observed discrepancies between some molecular divergence time estimates and the fossil record, as well as time-scale dependence of clock rates. Such effects may be direct (i.e. actual increase in clock rates) or indirect (i.e. changes in data availability that alters the observed rate). However, little is known about the relationships between Bayesian phylogenetic clock rates and mass extinctions.

The evolutionary model underlying the morphological clock is simple [26] and morphological data may be limited in number compared with molecular data and defined more subjectively. Despite these weaknesses, the use of the morphological clock enables one invaluable experimental design that is impossible with molecular data, i.e. short time spans from deep time can be studied by the morphological clock. Molecular rate estimates are based on the total duration of a living clade and thus represent an average over a long time span. Thus, to study time-scale dependence of evolutionary rates with molecular data, it is necessary to compare across different extant clades, some nested inside others. By contrast, with fossil data, the evolutionary rate in a single clade can be estimated at various time scales (e.g. the first 10, 20 or 30 Myr of its evolution), allowing time-scale dependence of evolutionary rates to be studied using a single clade, thus minimizing some phylogenetic biases.

This method is also useful in estimating divergence time values. To estimate the divergence time of an old clade that appeared, say, 250 Ma, it may be reasonable to use the evolutionary rates of the species that were diversifying early in the clade's history, rather than those from much later descendants. Reversing the perspective, the evolutionary rates of Recent animals are best studied based on these animals themselves, not their distant descendants that will appear some 250 Myr in the future.

The purpose of this study is threefold: (i) to examine whether and how the morphological evolutionary rate and divergence time estimates of a single clade may be biased by adding later and later clade members to the data; (ii) to test the validity of different rates using high-resolution stratigraphic confidence intervals; and (iii) to use such information to improve divergence time dating. We then discuss the impact of mass extinction on divergence time estimation based on the outcome. To address these questions, we used Ichthyosauromorpha, a clade of marine reptiles whose fossil record starts a few million years after the end-Permian mass extinction with a rapid diversification [27,28]. It presents one of the best-documented examples of adaptive radiation, where early evolutionary rates are expected to be higher than the average for the entire clade. It also holds the oldest fossil record of all Mesozoic marine reptiles, dated at 248.8 Ma, allowing us to specify the time of origin of these reptiles.

2. Material and methods

(a) Stratigraphic confidence interval

We calculated the stratigraphic confidence intervals [1] based on our field records at Majiashan, Chaohu, Hefei City, Anhui Province, China. We have been excavating marine reptile fossils at Majiashan since 2010 and collected more than 50 specimens from 13 beds distributed across a span of 223 beds (beds 594–816), representing an interval of about 1.2 Myr (248.8–247.7 Ma) within the mid-to-late Spathian (Early Triassic). Ichthyosauromorpha were found in 11 of the 13 beds, while *Chaohusaurus* was present in 10 (figure 1). The beds at Majiashan had been dated to the closest 0.1 Myr or finer, thanks to astrochronological cycles detected in carbon isotopes [27]. This temporal resolution is very high for Triassic fossils, given that most of them are only dated to geological substages, which are about 3 Myr long on average [29]. There are eight extensive outcrops in the area, repeatedly exposing the same sequence of the Lower Triassic that are continuous and complete. They are well exposed because of limestone quarrying and correlated with each other down to the level of individual beds. The outcrops yielded the oldest fossil of ichthyosauromorph in the world, dated at 248.8 Ma. This specimen belongs to the ichthyopterygian *Chaohusaurus*. The same outcrops have yielded the only specimens of the most basal ichthyosauriforms, *Cartorhynchus* [28] and *Sclerocormus* [30]. These and other most basal ichthyosauromorphs are known exclusively from South China, where Majiashan has the best stratigraphic record reaching the oldest level. Ichthyosauromorph fossils are also found in other parts of the world, but they are not as basal in the phylogenetic tree, and their ages are less precisely known [31]. Therefore, Majiashan arguably provides the best rock sequence for examining the stratigraphy of the oldest Ichthyosauromorpha.

Two species of the ichthyopterygian genus *Chaohusaurus*, namely *Chaohusaurus chaoxianensis* and *Chaohusaurus geishanensis*, occur in these rocks, together with *Chaohusaurus* sp. that

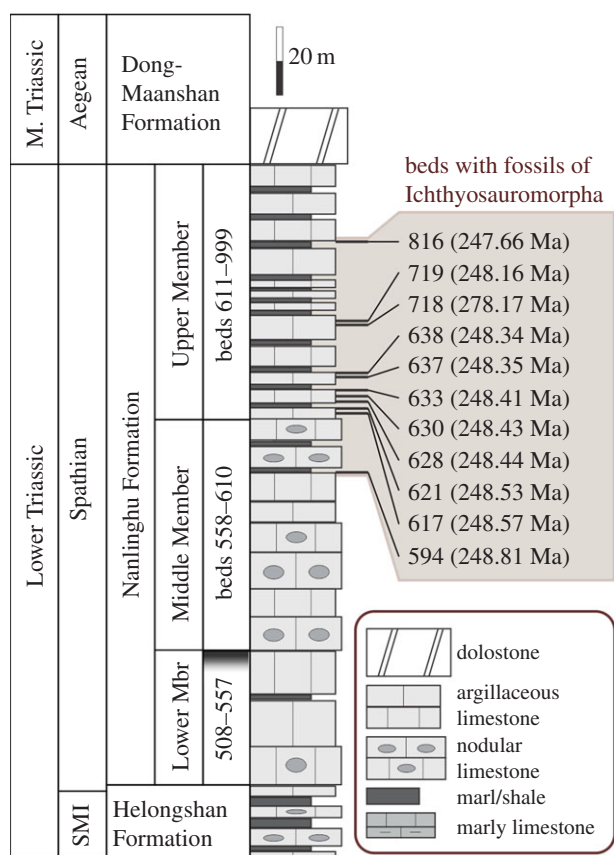


Figure 1. Simplified stratigraphic column based on four sections of Majiashan correlated at the bed level. A list of beds with ichthyosauromorph fossils, with their approximate ages and position within the column, is given. All but bed 719 yielded *Chaohusaurus*. *Cartorhynchus* is from bed 633, and *Sclerocormus* bed 719.

can be identified as one of the two but clearly not the third and younger species of *Chaohusaurus*, *Chaohusaurus zhangjiawanensis*, that is found elsewhere. They form a clade inside *Chaohusaurus* and will be referred to as the Majiashan *Chaohusaurus* clade hereafter.

The ichthyosauromorph specimens are mostly from the bottom part of the Upper Member of the Nanlinghu Formation, but the oldest occurrence is from the top part of the Middle Member. The formation comprises alternations of carbonate and clastic rocks, with thickening of carbonate beds towards the bottom. The proportion between the two types is at about the average for the entire formation in the stratigraphic range of Ichthyosauromorpha. The specimens are usually found at the boundary between the two rock types, embedded in either the carbonate or clastic side. Given that there are approximately as many carbonate/clastic boundaries as the bed number, it is reasonable to assume that the chances of fossil discovery, per bed, are almost uniform throughout the formation.

We used the classical confidence interval *sensu* Marshall [1], given that the variations of fossil discovery potential in the outcrops are poorly known. We tried using both the raw bed counts and time bins as the base data for the interval calculations. We calculated the stratigraphic confidence intervals for the Majiashan *Chaohusaurus* clade ($n = 43$ individuals) and Ichthyosauromorpha ($n = 45$ individuals). See below for suitability of the latter choice. With the time-bin approach, we used the bin width of 0.1 Ma, and found that six out of 12 bins contained *Chaohusaurus* occurrences (the numbers were the same for Ichthyosauromorpha).

We consider only the results for the smaller group, the Majiashan *Chaohusaurus* clade, to be accurate. This is because the method has an inherent bias that probably compromises the

result for the more inclusive clade. That is, it systematically results in narrower confidence intervals in a more inclusive clade than in a smaller clade nested within, when the smaller clade has the total stratigraphic span that equals that of the larger more inclusive clade in the data being analysed. This tendency is rooted in the way the confidence interval is calculated. It, of course, is unreasonable because a more inclusive clade must have emerged earlier than the smaller clades that are nested in it. The Majiashan *Chaohusaurus* clade, on the other hand, is suitable for the calculation. They are represented by a high number of skeletons ($n = 43$) for this kind of fossil clade. They are almost exclusively known from Majiashan with only a few exceptions, while the age of the specimens from elsewhere are comparable to the youngest known from Majiashan. Thus, the stratigraphic range at Majiashan is expected to be a reasonable estimate of the range for the clade. Therefore, the comparison between the stratigraphic confidence interval and Bayesian divergence time estimates is best performed using the Majiashan *Chaohusaurus* clade.

The geological timescale in the Early Triassic continues to be revised. We followed Song *et al.* [32] and used 247.2, 250.6, 251.1, 251.8 and 252.17 Ma for Anisian/Spathian, Spathian/Smithian, Smithian/Dienerian, Dienerian/Griesbachian and Griesbachian/Changhsingian boundaries, respectively. The same paper employed the age of 252.28 Ma for the end-Permian mass extinction.

It is also possible to calculate the stratigraphic confidence interval based on the number of geological formations with and without Ichthyosauromorpha through time [29,33]. This alternative approach, however, is unsuitable for the present case because of its low temporal resolution. The significant figure, a basic principle of statistics, mandates that the output from a statistical analysis cannot have finer units than the input. In the case of the confidence interval based on geological formations, the input data are compiled with one geological formation as the time unit, although one geological stage or substage may be used instead. The output needs to be rounded to the unit of the input data. Then, if we apply this method to our case, even a unit margin of error, with the length of, say, one average substage, would place the confidence limit for the origin of Ichthyosauromorpha in the Permian (Triassic substages are 3.2 Myr long on average). The use of geological formations or stages would usually give even larger margins. Clearly, such a low temporal resolution does not allow an effective test of our hypothesis. Another problem of this approach is the unrealistic simplification during data compilation. An approximation is made that if a fossil is present in a geological formation, then the whole formation contains that taxon. This procedure overestimates the antiquity of every taxon in the data unless a fossil occurs in the bottom bed of a geological formation. In our case, the Nanlinghu Formation does not contain any marine reptile fossil for the first 1.5+ Myr. Then, considering the Nanlinghu Formation to be 'positive' for ichthyosauromorph occurrence would automatically add 1.5 Myr to its antiquity. This margin is already larger than the stratigraphic interval that we obtained from our bed-based analysis.

(b) Bayesian morphological clock

We combined the morphological taxon-character matrices for Ichthyosauriformes [30] and Hupehsuchia [34] to build a single matrix for the entire Ichthyosauromorpha. This combined matrix, given in the electronic supplementary material, Dataset S1, was used in all analyses.

We constructed submatrices of the combined matrix based on stratigraphy. For example, the smallest submatrix contained only the Early Triassic taxa ($n = 14$), representing the earliest evolution of the clade that had unfolded by the end of the Early Triassic, or during the first 3.4 Myr of their fossil record. Only

one lineage out of the 14 species survived into the Middle Triassic; therefore, the omission of later species minimally decreases the phylogenetic resolution of the Early Triassic forms. Larger submatrices were subsequently built by adding later and later segments of the evolution of the clades in the matrix. The phylogenetic tree of Ichthyosauromorpha is largely ladder-like and shows a strong correlation with stratigraphy, minimizing the loss of information through these segmentations, as in the case made above for the smallest submatrix. Lists of taxa included in each submatrix are given in the electronic supplementary material, table S1.

Each submatrix was analysed using MrBAYES v. 3.2.6 [35,36], with variable character coding and using the γ distribution to sample rates. Each tip taxon was time calibrated using a uniform distribution within a range containing the geological substage of its oldest occurrence (electronic supplementary material, table S2). We also tried to extend the range to include the geological substage before the first substage, but the result was not compatible with the stratigraphic confidence interval, and therefore considered inapplicable. For four species from Majiashan, for which a more precise dating of the oldest fossil is available, we used those ages for the younger limit of the distribution. The values were 248.2 for *Sclerocormus*, 248.4 for *Cartorhynchus* and 248.8 for the two species of *Chaohusaurus*. All node ages were left unconstrained because the oldest fossil age of the group is already included in the tip data. The base clock rate was assumed to follow a lognormal distribution with a standard deviation of 0.5 and mean value of -5.36 , which was calculated from the mean number of character changes per million years in the most parsimonious trees. Clock rates were assumed to vary among branches according to the independent gamma rate model, with `igrvarpr` set at `exp(1)`. Fossilized birth–death priors were used for the branch length distribution, and fossil-tip sampling strategy was assumed. Priors for the parameters for the fossilized birth–death process were set in the following manner: `sampleprob` as 0.1, `fossilizationpr` as `beta(1,150)`, `speciationpr` as `exponential(1)` and `extinctionpr` as `beta(1,1)`.

We constrained the tree topology, but not branch lengths, across all analyses for consistency in analytical settings because different topologies may result from various submatrices, disabling a comparison across submatrices. We used the majority consensus tree of the most parsimonious trees from the complete dataset as the reference tree after arbitrarily dichotomizing it (figure 2; electronic supplementary material, Data S2). PALEOTREE [38] was used to convert the constraint tree topology to MrBAYES commands. The use of the topology constraint resulted in much smaller split frequencies than the commonly used threshold value of 0.01, thus prohibiting the use of split frequencies to judge convergence. To optimize branch lengths, we ran 5 million generations of optimization in each analysis. Four runs, each containing four chains, were run per analysis.

When using fossil-only datasets, MrBAYES v. 3.2.6 truncates the divergence ages, so that the youngest taxon in the data is placed near the modern time plane (i.e. age = 0 or nearby). We converted these tentative age values to geological ages by hanging the mean of the posterior age estimates at the mean of the prior age ranges. For example, with the Spathian-only dataset, the mean of the prior ages ranges was 249.1 Ma, while the resulting posterior age estimates had a mean of 1.24 Ma. The posterior age estimate of, say, *Cartorhynchus* was 1.96, so its geological age was calculated as $1.96 + 249.1 - 1.24 = 249.8$ Ma. We confirmed the validity of this hanging scheme by a simple experiment of removing the tip-age constraint from one of the taxa in a large dataset (i.e. the taxon is considered Recent by the software). It showed that the mean of the posterior ages for the taxa in the data except the one considered 'Recent' matched that for the prior age ranges for the same set of taxa.

3. Results

(a) Stratigraphic confidence interval

The older confidence limit (95%) of the stratigraphic range of the Majiashan *Chaohusaurus* clade was estimated to be 249.8 Ma using 0.1 Ma time bins. The use of the raw rock bed data rather than time bins resulted in a slightly deeper confidence limit of 250.2 Ma (bed 506). These values indicate that the fossil record of the Majiashan *Chaohusaurus* clade is not expected to extend back to the Smithian (251.8–250.8 Ma). The corresponding confidence limits for Ichthyosauromorpha were 249.8 and 250.1 Ma (bed 516), respectively, but see Methods.

(b) Bayesian morphological clock

The Bayesian morphological clock suggested various divergence times for the Majiashan *Chaohusaurus* clade depending on the stratigraphic extent of the dataset. The youngest divergence time estimate was given by the smallest dataset, containing only the earliest evolutionary history of Ichthyosauromorpha, in the Spathian. The divergence time became deeper and deeper as the data became more temporally inclusive, i.e. as later and later parts of the ichthyosauromorph evolution were added (figure 3; electronic supplementary material, table S3).

The youngest divergence time estimate for the Majiashan *Chaohusaurus* clade was 249.8 (246.8–250.6) Ma, and that for Ichthyosauromorpha 251.5 (246.2–253.4), suggesting the emergence of the clade after the end-Permian mass extinction, probably during the Smithian. See the electronic supplementary material, table S3 for other results. Associated posterior Bayesian statistics are given in the electronic supplementary material, table S4.

4. Discussion

Of the various Bayesian divergence time estimates for the Majiashan *Chaohusaurus* clade, only one passed the test by the stratigraphic confidence interval, i.e. the result from the smallest dataset that contained only the first 3.4 Myr of the fossil record of Ichthyosauromorpha. The corresponding mean estimate of divergence time by the Bayesian morphological clock was 249.8 Ma, falling within the confidence limit resulting from the stratigraphic data (249.3–250.2 Ma, depending on the binning scheme of the data). Note that the deeper 95% interval from this best Bayesian estimate (250.6 Ma) is outside of the range of the stratigraphic confidence interval, suggesting that the mean Bayesian estimate, rather than its deeper 95% interval, is a more suitable estimate of the lower limit of divergence time. Based on this principle, we infer that the Majiashan *Chaohusaurus* clade most likely emerged between 250.2 and 248.8 Ma, the latter representing the age of its oldest fossil record, whereas Ichthyosauromorpha originated between 251.5 and 248.8 Ma, during the Olenekian (i.e. the Smithian and Spathian combined). Given that other marine reptile clades have slightly younger fossil records and that Ichthyosauromorpha established diverse feeding guilds before other marine reptiles [31], there is no evidence to support Mesozoic marine reptiles emerging before 251.5 Ma at this point.

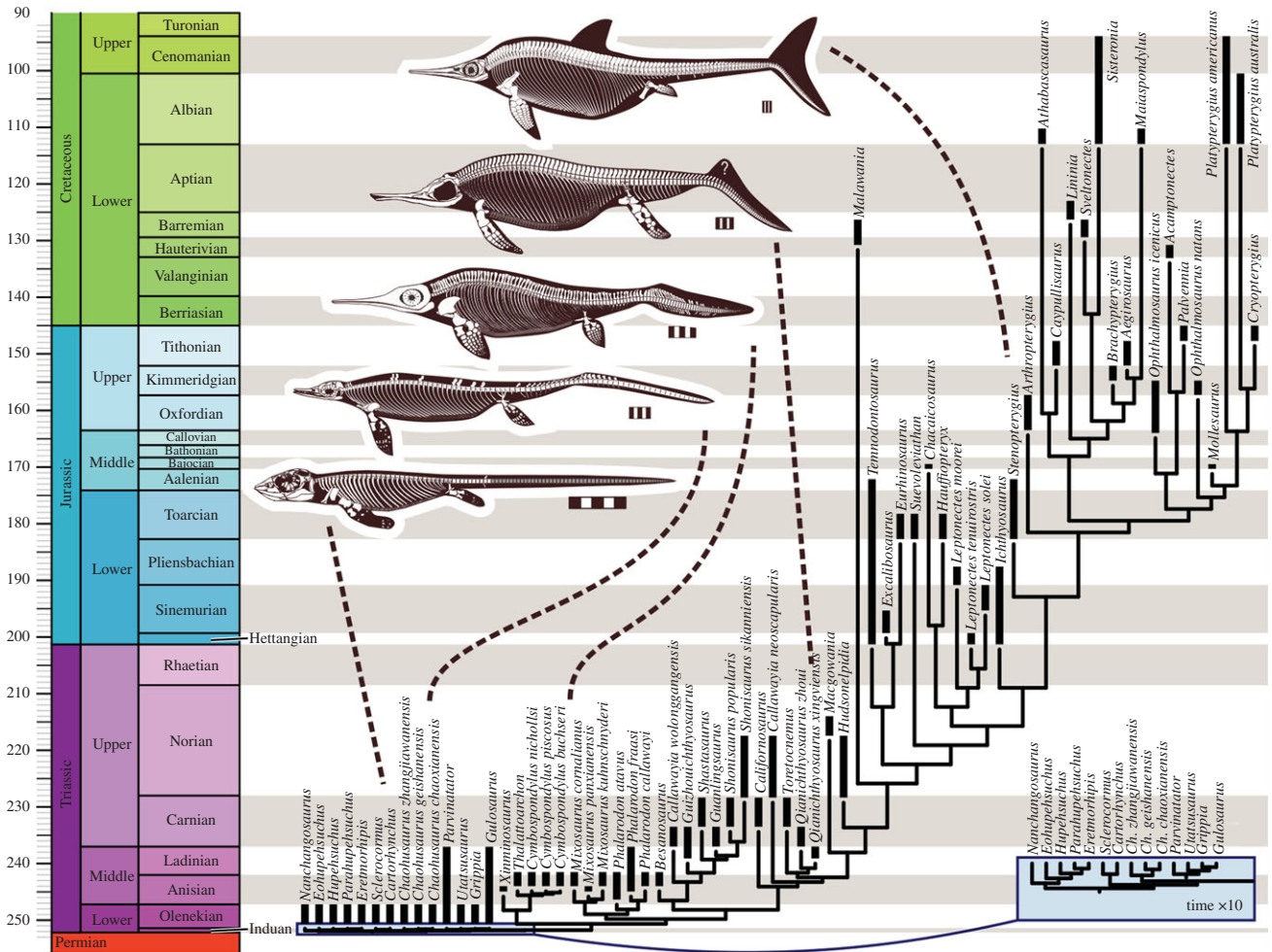


Figure 2. Phylogenetic tree of Ichthyosauromorpha used as the constraint tree, superimposed on a stratigraphic column. The stratigraphic range of each taxon is calibrated to the substage level, although some taxa may contain fossil records without strict stratigraphic calibration that makes their ranges unusually long. Node ages were estimated based on the Bayesian morphological clock from the smallest possible data matrix used in this study to contain the node, to avoid time-scale dependence. Strap was used for the stratigraphic column [37]. Skeletal reconstructions of *Cartorhynchus*, *Chaohusaurus*, *Mixosaurus*, *Qianichthysaurus* and *Stenopterygius* (from bottom to top) have been added to outline the evolution of the clade. Scale bars are 5 cm. (Online version in colour.)

Our preference for the youngest divergence time is justified not only by the stratigraphic confidence interval but also by the logic that the initial evolutionary rate during the first radiation should dictate the time it took for the divergence. It is also supported by the timing of the end-Permian mass extinction and the environmental changes that followed. Air-breathing predators are not recorded from the margins of open seas before this extinction, whereas change in ocean chemistry after the mass extinction probably encouraged marine invasions by air-breathers [27]; note, however, that the last common ancestor of Ichthyosauromorpha may have been amphibious or terrestrial. Moreover, the average sea surface temperature was lower in the mid-to-late Permian than in the Early Triassic, which started in a greenhouse condition resulting from the global warming associated with the end-Permian mass extinction [39]. Given that marine reptiles are physiologically constrained to prefer warm waters [40], it is more likely for marine invasion to have occurred in the Triassic than in the Permian. The only saltwater reptiles from the Permian, Mesosauridae, lived during a short-term warming [41] in the late Artinskian (Early Permian) in an enclosed sea [42] and quickly became extinct as the temperature dropped. Additionally, deeper divergence time estimates, resulting from more temporally inclusive datasets, point to as far back as the Mid-Permian.

This divergence time is unreasonably early because fossil ichthyosauromorphs would almost surely have been found over the long time interval representing six geological stages of the Late Permian, especially in China where a Late Permian section, rich with vertebrate fossils, has been well studied. There are several marine vertebrate beds during those time periods but without marine reptiles, including those near the Smithian/Spathian boundary in Majiashan [43]. It is possible to analyse such presence/absence of a taxon across geological formations, but its time resolution is low [29] and therefore unsuitable to our case (Material and methods).

There is a clear tendency for the Bayesian estimates of the morphological rate of evolution in Ichthyosauromorpha to become slower and slower as later and later parts of its evolution are added to the data (figure 4a)—a linear relationship is observed between the time span represented in the data and the reciprocal of the clock rate ($r^2 = 0.980$). As mentioned earlier, the fastest rate of evolution is inferred from the smallest dataset representing only the Spathian, when Ichthyosauromorpha had their initial adaptive radiation within a short time span. This initial fast rate seems to be diluted by averaging it with slower rates from later parts of their evolution, as they are added to the data. Our result demonstrates that this bias is correlated with the time span

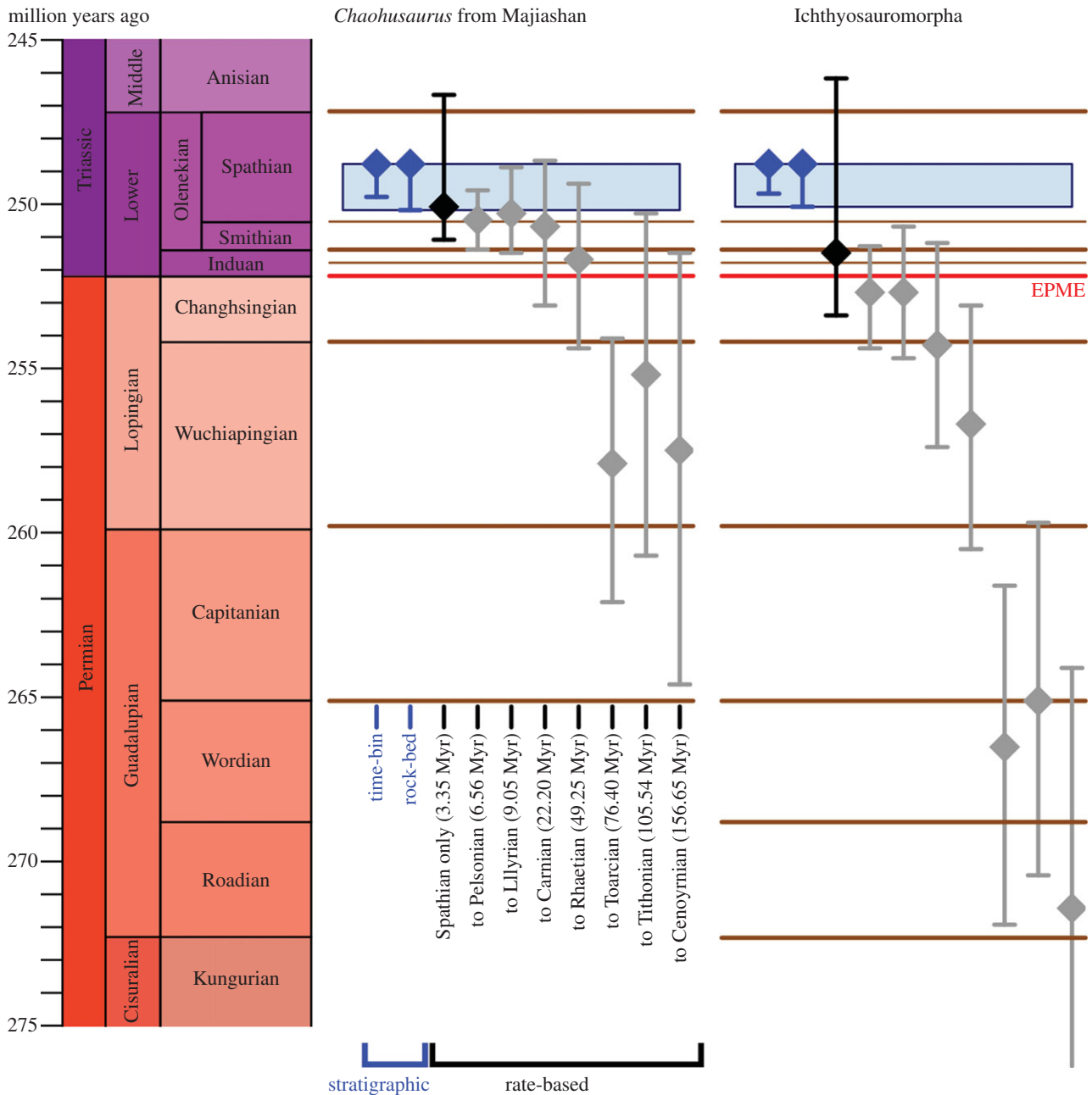


Figure 3. Stratigraphic confidence intervals, Bayesian divergence time estimates and their data-dependent variations for Ichthyosauromorpha and the two species of *Chaohusaurus* from Majiashan. Blue colour indicates stratigraphic intervals. Black colour is for the best Bayesian estimate. Grey colours are for Bayesian estimates that did not pass the test by the stratigraphic confidence intervals. Blue rectangles denote the time range that are compatible between the stratigraphic confidence interval and the best divergence time estimate, based on the Spathian only. Red line and EMPE stands for the end-Permian mass extinction. Myr stands for million years. (Online version in colour.)

represented in the dataset, rather than the antiquity of the data points (i.e. how old a clade is), because the age of the oldest data point is invariant.

The divergence time estimates also became older as the clock rate slowed (figure 4*b*). There is an approximately linear relationship ($r^2 = 0.954$) between the estimated divergence time and the reciprocal of the clock rate, although a disjunction seems to emerge when the post-Triassic data are added to the Triassic ones (green arrow), possibly indicating a change in evolutionary dynamics after the Late Triassic extinctions during marine regressions [44]. Similar changes are also observed in relation to other parameters, including that between the net speciation rate and clock rate (figure 4*c*). Given that the smallest dataset gave the only divergence time estimates that are compatible with the

stratigraphic confidence limits, we infer that the divergence time is best estimated based on the earliest evolutionary rates of the clade in question, probably representing the rates during the initial radiation, rather than the average rates over a long span of time. This logic is reasonable as long as there are sufficient data to estimate the earliest rate of evolution in the clade in question. The fossil records of many groups may be poor near the base and therefore devoid of such data, especially in terrestrial clades, in which case it may be necessary to use longer-term rates, noting the limitation.

Potential biases of our results include incomplete representation of taxa in the data owing to biases in fossilization, fossil collection and taxon sampling. Species sorting, *sensu* Jablonski [45], may add to the fossilization

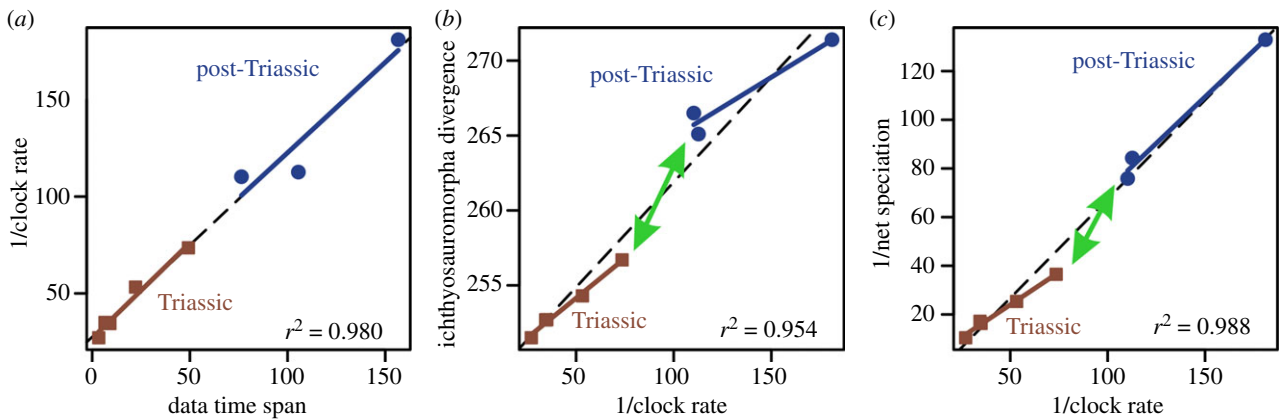


Figure 4. Bivariate relationships between pairs of posterior Bayesian statistics and data time span. (a) The reciprocal of the posterior morphological clock rate versus the time span represented by the data, clearly showing time-scale dependence of morphological clock rates. (b) Median posterior divergence time estimates for Ichthyosauroomorpha versus the reciprocal of the posterior morphological clock rate, suggesting time-scale dependence of divergence time. (c) The reciprocal of the posterior net speciation rate versus the reciprocal of the posterior morphological clock rate. Brown and blue colours indicate whether the data used included only the Triassic taxa or a mixture of Triassic and post-Triassic taxa. Lines are ordinary least-square regression lines. The broken regression line is for the entire data points on respective plots. Green arrows point to the disjunction of the regression lines discussed in text. (Online version in colour.)

bias if a species was present during a given period but became extinct before the time horizon when fossiliferous rock layers were formed, without leaving fossils. Also, neglect of unusually large changes in extinction and speciation rates at historically specific points of time, such as the end-Permian and end-Triassic mass extinctions, may create bias. Some of these factors are incorporated in the clock model of MRBAYES using specific statistical distributions. Unique events in Earth history, such as individual mass extinction events, may fall outside the expected values from a statistical distribution based on normal conditions. Emerging methods may ease the problem in the future when the timing of time-simultaneous changes across taxa, such as a mass extinction, is known [46].

It is uncertain how pertinent our results and inferences are to molecular divergence time estimation, given that the model of character evolution and the nature of the data are different between morphological and molecular clocks. Advancements in molecular clock models are reducing the time-scale-dependent bias, at least to some extent [12]. However, at the mechanistic level, some of the potential causes of the time-scale dependence of morphological rates are analogous, at different scales, to those that have been proposed to explain a similar time-scale dependence of molecular rates—they mostly concern the loss of information on variations in the past, whether intraspecific or interspecific, and natural selection and species sorting could affect both types of data. For example, if the underlying clock rate was fast, but mutations and the resulting shape disparity were frequently removed through natural selection and species sorting, the apparent clock rate inferred from the genes and

fossils that survived until present may be slow, despite the best compensation effort through modelling based on the same surviving data, as implied by some ancient DNA data [47]. However, after a mass extinction, when natural selection and species sorting are weaker, more mutations may survive to be included in the data to make the apparent clock rate closer to the underlying rate. If our findings apply to divergence time estimates based on molecular data, the lack of molecular data on early members of a clade implies that divergence times based on the fossil record and molecules will often differ, especially for old clades.

Data accessibility. All data used in this study are available in Material and methods; electronic supplementary material or reference [18].

Authors' contributions. R.M. conceived the project, as a part of a larger project co-conceived with D.-y.J. and A.T., R.M. also designed and ran the analysis, and drafted the paper. D.-y.J. compiled the field data. A.T. noticed the patterns of vertebrate fossil occurrence at Majiashan. R.M., D.-y.J., A.T., C.J. and J.-d.H. all revised the manuscript, and contributed to the fieldwork that gave rise to the stratigraphic data.

Competing interests. We declare we have no competing interests.

Funding. National Geographic Society Committee for Research and Exploration (no. 8669-09) to R.M., Project 40920124002 and 41372016 from the National Natural Science Foundation of China to D.-y.J., Project 123102 from State Key Laboratory of Palaeobiology and Stratigraphy (Nanjing Institute of Geology and Palaeontology, CAS) to D.-y.J. and Project 201511054 from the Ministry of Land and Resources of China to J.-d.H., Y.-C.H., R.M. and D.-y.J.

Acknowledgements. We thank Geerat J. Vermeij for his suggestions on two earlier versions of the manuscript. We are also grateful to him and Alyssa Henry for proofing the language.

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