

## Comment

# Nuclear DNA does not reconcile 'rocks' and 'clocks' in Neoaves: a comment on Ericson *et al.*

The discrepancy between fossil- and molecular-based age estimates for the diversification of modern birds has persisted despite increasingly large datasets on both sides (Penney & Phillips 2004). For the purpose of addressing this discrepancy, Ericson *et al.* (2006) recently generated a significant neoavian dataset that is well represented by taxa (87 species comprising 75 traditional families), characters (five nuclear genes) and fossil calibrations ( $n=23$ ). The divergence times reported in this study are by far the youngest yet reported from genetic data. These authors conclude that there is no reliable molecular support for extensive diversification of Neoaves in the Cretaceous. While an increased agreement with the fossil record is encouraging (and, indeed, sought after), we find a number of problems with their study that calls this conclusion into question.

Our first concern with this paper involves the particular fossils used to calibrate and constrain estimated divergence times. Fossils are of fundamental importance in estimating dates with molecular sequence data, and care should be taken that they are taxonomically and stratigraphically well identified. While the fossils used in Ericson *et al.* (2006) appear to fit these criteria, we nevertheless take issue with the particular fossils used. First, Ericson *et al.* (2006) use a stem group galliform fossil (53 Myr; Mayr & Weidig 2004) to date the divergence between Galliformes and Anseriformes, despite the fact that an older (66 Myr), and therefore more appropriate, fossil anseriform calibration exists (Clarke *et al.* 2005). Ericson *et al.*'s (2006) estimate of the age of the Galliformes–Anseriformes split is approximately 53 Myr, 13 Myr younger than the minimum age definitively known from the fossil record (Benton & Donoghue 2006). Second, for the (required) fixed calibration, they use a 47.5 Myr stem group representative of Trochilidae to mark the splitting of hummingbirds from other Apodiformes. No rationale is given explaining why this fossil was adopted, and we note that an older (62 Myr), more derived and hence more appropriate fossil is established from the stem of Sphenisciformes (Slack *et al.* 2006). Regardless, owing to the importance of the single fixed constraint, alternatives should have been considered. Third, the authors impose a maximum constraint of 95 Myr on

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the age of Neoaves, despite the fact that earlier dates have been published (e.g. van Tuinen & Hedges 2001; Pereira & Baker 2006). Finally, one of their fossil calibrations (stem Strigiformes) is uninformative for dating purposes, as it is superseded by an equally old (55 Myr) but more derived fossil (Coliidae).

Our second concern involves the reliance on PATHd8 for estimating lineage ages. Ericson *et al.* (2006) also used the program r8s (Sanderson 2003), but dismissed these results simply because these dates are older than those generated by PATHd8 (although the older r8s dates are consistent with previous molecular-generated dates). The inferred dates from r8s directly contradict their claim of an absence of neoavian diversification in the Cretaceous. Agreement with the fossil record, while satisfying in terms of congruence, is not a sufficient criterion to arbitrate between sets of dates generated by different methods. Rather, arbitration should rely upon the performance of methods on both empirical and simulated data, and PATHd8 has yet to be tested in this way. To compare their PATHd8 results with those from a well-vetted program, we reanalysed the data of Ericson *et al.* (2006) using a Bayesian modelling of rate evolution (Thorne & Kishino 2002) and the revised calibrations outlined previously (see electronic supplementary information for methods). Contrary to their results, we find evidence for substantial diversification of Neoaves in the Cretaceous (figure 1).

Finally, and most importantly, nowhere do Ericson *et al.* (2006) mention any error intervals on their dating estimates. Given the proximity of many nodes to the K–T boundary, confidence intervals on age estimates would cross into the Cretaceous and render their conclusion untenable. Error estimates are easily generated using either non-parametric bootstrapping or considering a posterior distribution of trees. As error is inherent in each step of molecular dating (sequences, alignment, fossils, trees, etc.), the lack of error calculation is disturbing and undermines their ultimate assertion. When incorporating error intervals in our reanalysis, 24 basal neoavian divergences are restricted to the Cretaceous (figure 1, green bars). Of these, 15 lead directly to extant families. While the addition of further family representatives will undoubtedly break up some of these branches (forming crown clades), a Tertiary origin for much of Neoaves is clearly rejected.

Given the results of our reanalysis of the data of Ericson *et al.* (2006), the noteworthy problems attendant in their study and the plurality of genetic studies indicating a Cretaceous origin of modern birds, we respectfully disagree with their conclusion and find instead that there is no reliable molecular evidence against an extensive pre-Tertiary radiation of Neoaves.

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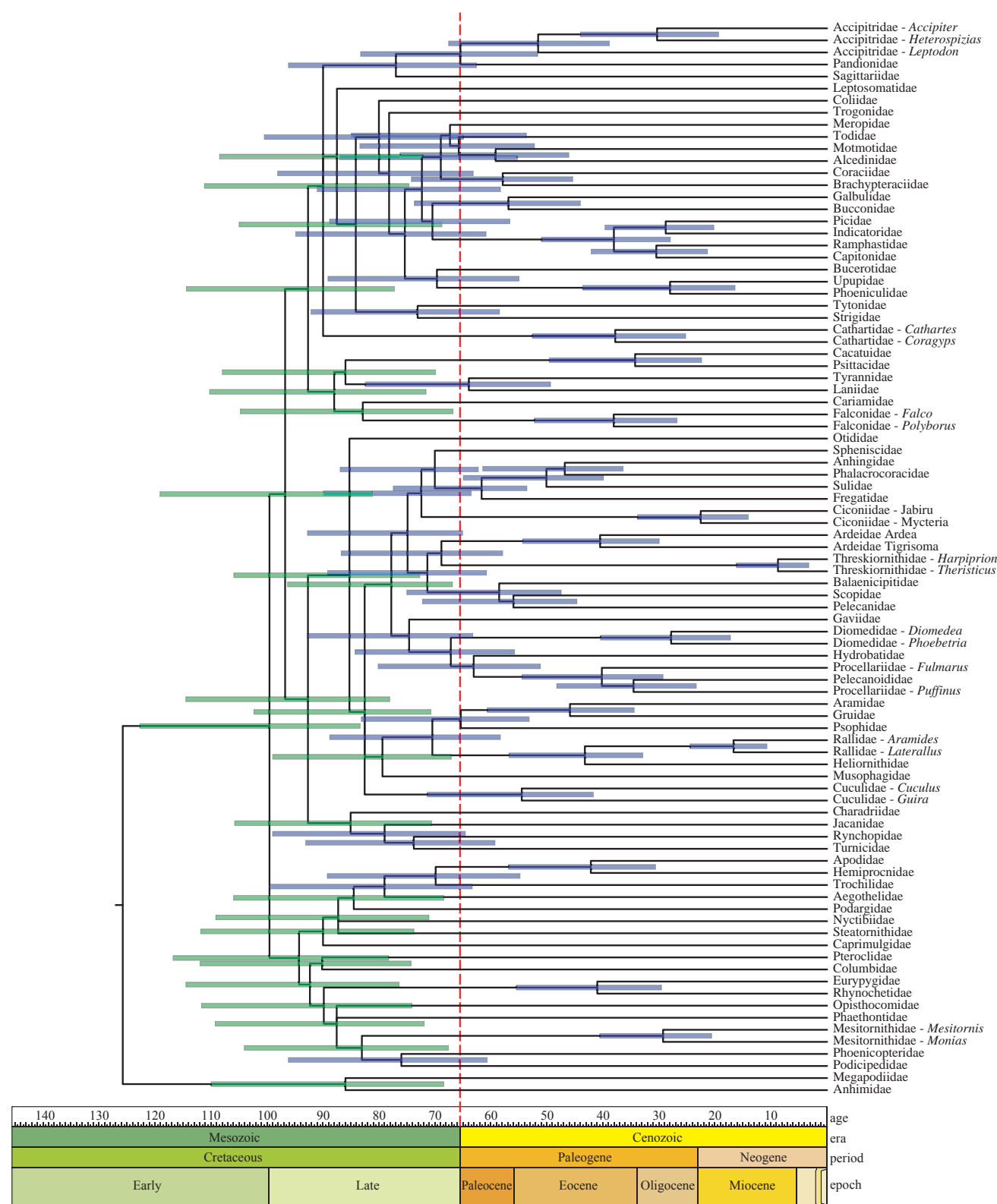


Figure 1. Chronogram for Neoaves estimated using a Bayesian modelling of rate evolution. The dashed vertical red line marks the K–T boundary. Error bars represent posterior probability (0.95) credible intervals (root node 104–105 Myr). An unambiguous ancient diversification of Neoaves is indicated by 24 credible intervals restricted to the Cretaceous (green bars).

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