



Calibrating the genomic clock of modern birds using fossils

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Wu et al. (1) conducted a phylogenomic analysis resulting in a new time frame for the diversification of modern birds, concluding that the rapid radiation of Neoaves occurred well before the Cretaceous–Paleogene (K–Pg) extinction event and that this catastrophic event did not affect their diversification dynamics. Here, we show that the divergence times obtained by Wu et al. were compromised by problems with their choices of fossils and calibration strategy.

Of the 20 fossil-based age constraints used by Wu et al., 11 are problematic. The only maximum constraint in the bird clade, applied to the crown age of Neornithes, was based on the oldest known fossil of *Ichthyornis dispar*. A fossil can provide a maximum bound for the age of a node if it is a direct ancestor of the node but the oldest fossil of a sister group constrains the minimum stem age, not the maximum age of the crown clade (Fig. 1A) (2, 3). Because *Ichthyornis* is neither a direct ancestor nor the sister group of Neornithes (4), its oldest fossil cannot directly inform age bounds for modern birds.

Ten minimum age constraints were also problematic. In eight, Wu et al. overlooked well-known fossils that are older and of higher quality, according to current best practices (8) (Table 1). For three of these, they overlooked fossils in the sister clade, which constrain the stem age as much as fossils in the focal clade (3). As a result, the underestimation of minimum ages ranged from –1.7 to 26.3 Ma across calibrations (Table 1). In the case of Aequornithes, the estimated divergence times were younger than the minimum ages indicated by two high-quality and precisely dated fossils (Table 1).

Despite minimum ages set too young, the age of Neornithes estimated by Wu et al. (130 Ma) was much older than even their assumed maximum bound of 94.3 Ma. Our maximum-likelihood analysis of clock-like coding sequences from Wu et al. resulted in similarly old dates when using their

minimum-maximum calibration constraints (Fig. 1B). In contrast, when we used information from calibration densities derived from the fossil record (5, 10), we found younger ages supporting a rapid radiation of Neoaves near the K–Pg boundary (Fig. 1C). We obtained similar results with different genomic data types and partitioning schemes (additional analyses available at <https://doi.org/10.5281/zenodo.11074217>) and using a more thorough Bayesian analysis of a larger phylogenomic dataset (9). Therefore, in addition to problems with the fossils, the old ages found by Wu et al. may be the result of using ill-defined minimum and maximum bounds which do not represent appropriately the calibration information that can be derived from fossils.

Therefore, the conclusion by Wu et al. that the rapid diversification of Neoaves occurred in the mid-Upper Cretaceous, with the K–Pg extinction event having little influence, is unsubstantiated. When information from the fossil record is used more thoroughly, a rapid radiation of modern birds is evident around the K–Pg boundary (5, 9).

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Table 1. Problematic minimum age calibration constraints in Wu et al. (1)

Calibration node	Minimum (Ma)		Problem
	Wu et al.	correct	
Incorrect minimum age:			
Stem Corvidae	7.2	17.2	Based on <i>Corvus larteri</i> [sic] (= <i>Miocorvus larteti</i> Milne-Edwards, 1871) from the middle Miocene of Sansan, France, age MN 6, thus 13.7 Ma minimum. Moreover, because no other member of the infraorder Corvidae was included, the “stem Corvidae” node in the tree is the most recent common ancestor of the infraorders Corvidae and Passerides, for which numerous older fossils exist, including <i>Kurrartapu johnguyeni</i> Nguyen, 2013 (Corvidae: Artamidae, 23.0 to 14.8 Ma) and <i>Certhiops rummeli</i> Manegold, 2008 (Passerides: Certhioidea, 19.5 to 17.2 Ma) (9).
Split suboscines - oscines	13.6	30.0	Based on <i>Miocitta galbreathi</i> Brodkorb, 1972, distal fragment of humerus referred to Corvidae. But, for the same node, there are numerous high-quality fossils (nearly complete skeletons) that are more than twice as old, such as the suboscine <i>Wieslochia weissi</i> Mayr and Manegold, 2006 from Frauenweiler (32 to 30 Ma), Germany (4, 5, 9).
Stem Psittaciformes	53.5	51.8	Based on <i>Pulchrapollia gracilis</i> Dyke and Cooper, 2000, which is more likely a stem member of Psittacopasseria, thus not constraining the stem of Psittaciformes, but instead can be constrained by the stem-Passeriformes <i>Eozygodactylus americanus</i> Weidig, 2010, from Green River Formation (51.8 Ma), USA (9).
Stem Coraciidae + Brachypteraciidae	51.6	54.6	Based on <i>Primobucco mcgrewi</i> Brodkorb, 1970, but <i>Septencoracias morsensis</i> Bourdon et al., 2016 from the Fur Formation (54.6 Ma), Denmark, is older (4, 9).
Stem Coliiformes	56.2	62.2	Based on <i>Sandcoleus copiosus</i> Houde and Olson, 1992, but <i>Tsidiyazhi abini</i> Ksepka et al., 2017 from the Nacimiento Formation (62.2 Ma), USA, is older (4, 9).
Stem Gruoidea	28.3	54.6	Based on <i>Parvigrus pohli</i> Mayr, 2005, but the stem age of Gruoidea is the crown age of Gruiformes, for which there are much older fossils such as <i>Pellornis mikkelsenii</i> Bertelli et al., 2011 from the Fur Formation (54.6 Ma), Denmark (4, 9).
Stem Apodiformes	51.6	54.6	Based on <i>Eocypselus rowei</i> Ksepka et al., 2013, but <i>Eocypselus vincenti</i> Harrison, 1984 from the Fur Formation (54.6 Ma), Denmark, is older (4, 9).
Stem Galliformes	51.6	65.7	Based on <i>Gallinuloides wyomingensis</i> Eastman, 1900, but the stem age of Galliformes is the crown age of Galloanseres, for which there are several older fossils such as the anseriform <i>Conflictio antarcticus</i> Tambussi et al., 2019 from the López de Bertodano Formation (66.0 to 65.7 Ma), Antarctica, extending its minimum bound to at least the K–Pg boundary (9).
Estimated age younger than oldest fossil:			
Stem Fregatidae	40.6	51.6	The estimated divergence between Fregatidae and Phalacrocoracidae was younger than a high-quality fossil in this clade: <i>Limnofregata azygosternon</i> Olson, 1977, from the Green River Formation, USA (4, 9).
Stem Sphenisciformes	45.7	60.5	The estimated divergence between Procellariiformes and Sphenisciformes was younger than the oldest fossil in this clade: <i>Waimanu manneringi</i> Slack et al., 2006, from the Waipara Greensand Formation, New Zealand (4, 9).

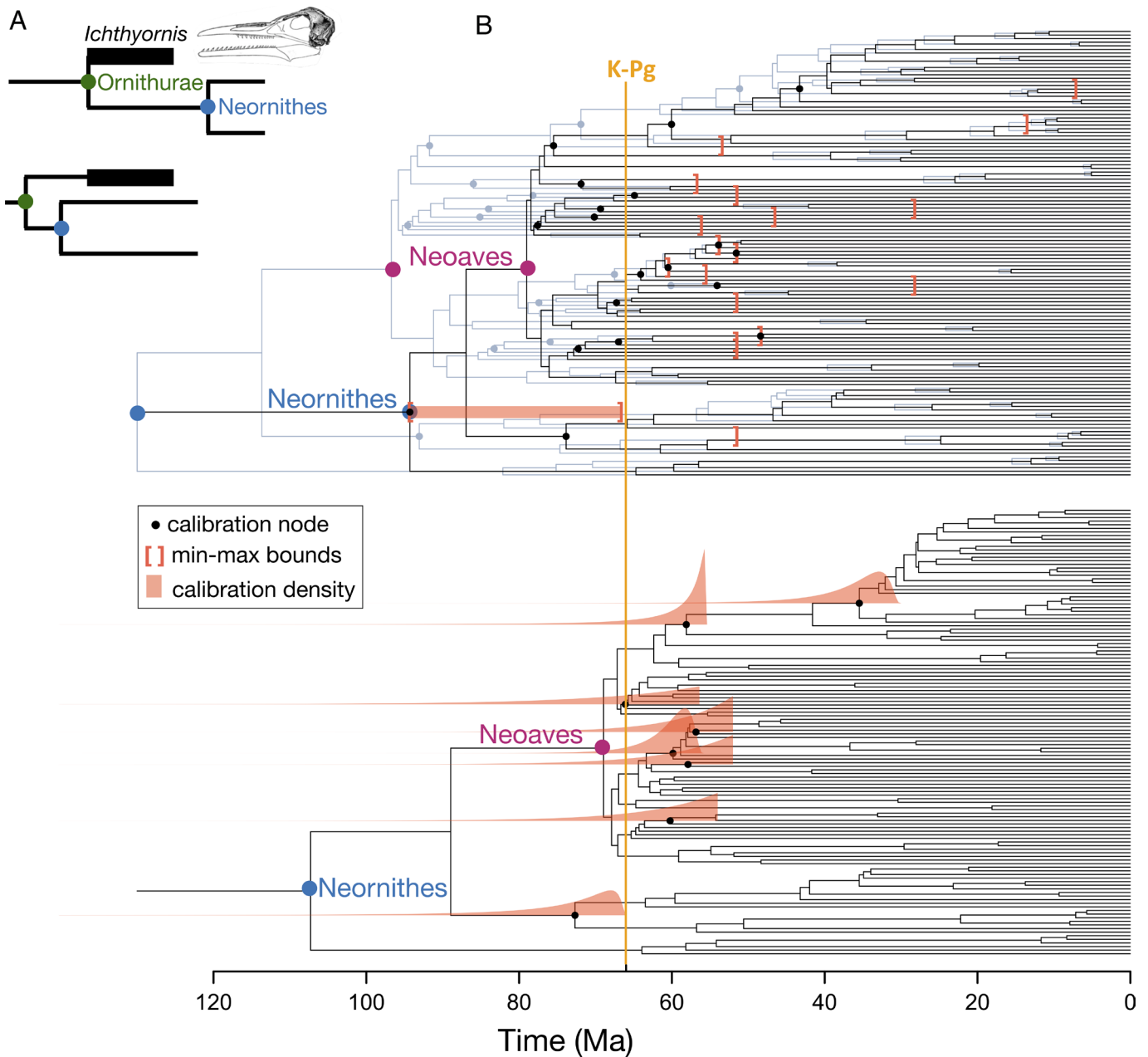


Fig. 1. Time-trees of modern birds from fossil calibrations. (A) *Ichthyornis* constrains the minimum possible age of Ornithurae because the clade cannot be younger than its oldest fossil (Top) but, not being a direct ancestor, *Ichthyornis* does not constrain the maximum possible age of Neornithes, which can be older than *Ichthyornis* (Bottom). (B) Top: Time-tree obtained by maximum-likelihood analysis based on calibration bounds (red brackets) from Wu et al. (1) (an alternative calibration using a 130 Ma maximum age constraint for Neornithes based on their results, is shown in light blue). Bottom: Time-tree based on the same maximum-likelihood tree but calibration information from ref. 5, using the medians of the calibration densities as fixed ages. The initial maximum-likelihood tree was generated by using IQ-TREE (6) to analyze the 1000 clock-like coding loci from Wu et al. (1), using the GTR + gamma + invariable sites model partitioned by codon position. Time-trees were obtained from the maximum likelihood tree by maximum-likelihood time-tree rescaling using a relaxed molecular clock with five discrete rate categories in the function *chronos* in R (7). The yellow line represents that Cretaceous–Paleogene (K–Pg) boundary. Illustration of the skull of *Ichthyornis* modified from O.C. Marsh 1886, Public Domain, via Wikimedia Commons. Sequence alignments, code, alternative analyses, and resultant trees are available at <https://doi.org/10.5281/zenodo.11074217>.

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