PNAS LETTER

Reply to Claramunt et al.: Robustness of the Cretaceous radiation of crown aves

Shaoyuan Wu[a,1], Frank E. Rheindt[b], Jin Zhang[c], Jiajia Wang[a], Lei Zhang[a], Cheng Quan[d], Zhiheng Li[e], Min Wang[e], Feixiang Wu[e], Yanhua Qu[f,1], Scott V. Edwards[g,1], Zhonghe Zhou[e,1], and Liang Liu[h,1]

Claramunt et al. (1) suggested our dating results (2) for a Cretaceous radiation of crown Aves may be inaccurate due to the inappropriate use of fossil calibrations. However, their criticisms stem from misinterpretations of fossil evidence and misconceptions of clock methodology, rendering their arguments baseless.

Claramunt et al. considered 8 of our 20 minimum bounds problematic and proposed alternative values based on different fossils, on average ~1.5 times older than ours. In many cases, it is debatable which team chose the more appropriate fossils as uncertainty surrounds the exact phylogenetic position of some of their choices (e.g., *Eozygodactylus, Tsidiiyazhi, Conflicto*) (3–5). In other cases (e.g., Fregatidae, Sphenisciformes), fossils they highlighted likely belong to older stem lineages, as we had explained for Fregatidae (2).

Ultimately, however, the choice over which set of fossils to use is inconsequential to their argument. Claramunt et al.'s eight alternative bounds are even older than ours and therefore corroborate our original results by pushing divergences further back in time. Similarly, their criticism of our use of *Ichthyornis* (94.3 Ma) as a maximum rather than minimum bound for crown Aves is impossible to reconcile with their conclusions, because their preferred usage pushes our estimates even further into the past. This prediction is confirmed by a new MCMCtree (6) analysis based on their eight alternative bounds and *Ichthyornis* as a minimum bound (Fig. 1).

Recent work involving the authors of Claramunt et al. employed 86.5 Ma as the maximum bound for crown Aves without supporting fossil evidence and included *Waimanu* (60.5 Ma) as the minimum bound for crown Aves (7), ignoring older Cretaceous fossils such as *Asteriornis* (66.7 to 66.8 Ma) (8) and *Vegavis* (68.4 to 69.2 Ma) (9, 10). Furthermore, a recent tip-dating analysis of avian fossil data arrived at an expected origination of ~100 Mya for major avian crown groups, broadly consistent with our results (11).

Claramunt et al. conducted a new analysis following the calibrations and methods proposed in their previous work (8), supporting a post-KPg (Cretaceous-Paleogene Boundary) radiation of crown Aves. Yet, their results cannot be replicated: incorporating the calibrations as much as the program allowed, we invariably obtained older Cretaceous dates for early avian divergences using MCMCtree (6) (Fig. 2). We concur with Referee 1 of their earlier work (8), who had warned that their "...calibration analyses... are very hard to understand and... impossible to replicate...."

Part of the discrepancy surrounding their younger divergences must be attributable to their unorthodox use of fossil dates. Our analysis involved time priors with soft bounds based on fossil calibrations (2). In contrast, Claramunt et al. used the median of subjectively estimated fossil distributions as fixed ages, which, due to the incompleteness of the fossil record, cannot be interpreted empirically to define prior bounds (1, 7).

Overall, Claramunt et al.'s conclusions are unsubstantiated because they selectively removed key fossil constraints and

Author affiliations: [a]Jiangsu Key Laboratory of Phylogenomics & Comparative Genomics, Jiangsu International Joint Center of Genomics, School of Life Sciences, Jiangsu Normal University, Xuzhou, Jiangsu 221116, China; [b]Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore; [c]School of Computer and Communication Engineering, Changsha University of Science & Technology, Changsha, Hunan 410114, China; [d]School of Earth Science and Resources, Chang'an University, Xi'an, Shaanxi 710054, China; [e]Key Laboratory of Vertebrate Evolution and Human Origins, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China; [f]Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; [g]Department of Organismic and Evolutionary Biology, Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138; and [h]Department of Statistics & Institute of Bioinformatics, University of Georgia, Athens, GA 30606

Author contributions: S.W., S.V.E., Z.Z., and L.L. designed research; S.W., F.E.R., Y.Q., S.V.E., Z.Z., and L.L. performed research; Y.Q. contributed new reagents/analytic tools; S.W., F.E.R., J.Z., J.W., L.Z., C.Q., Z.L., M.W., F.W., Y.Q., S.V.E., Z.Z., and L.L. analyzed data; S.W. conceived of the idea; F.E.R., S.V.E., Z.Z., and L.L. contributed to the writing and discussion; and S.W. wrote the paper.

The authors declare no competing interest.

[1]To whom correspondence may be addressed. Email: shaoyuanwu@post.harvard.edu, quyh@ioz.ac.cn, sedwards@fas.harvard.edu, zhouzhonghe@ivpp.ac.cn, or lliu@uga.edu.

Published September 16, 2024.

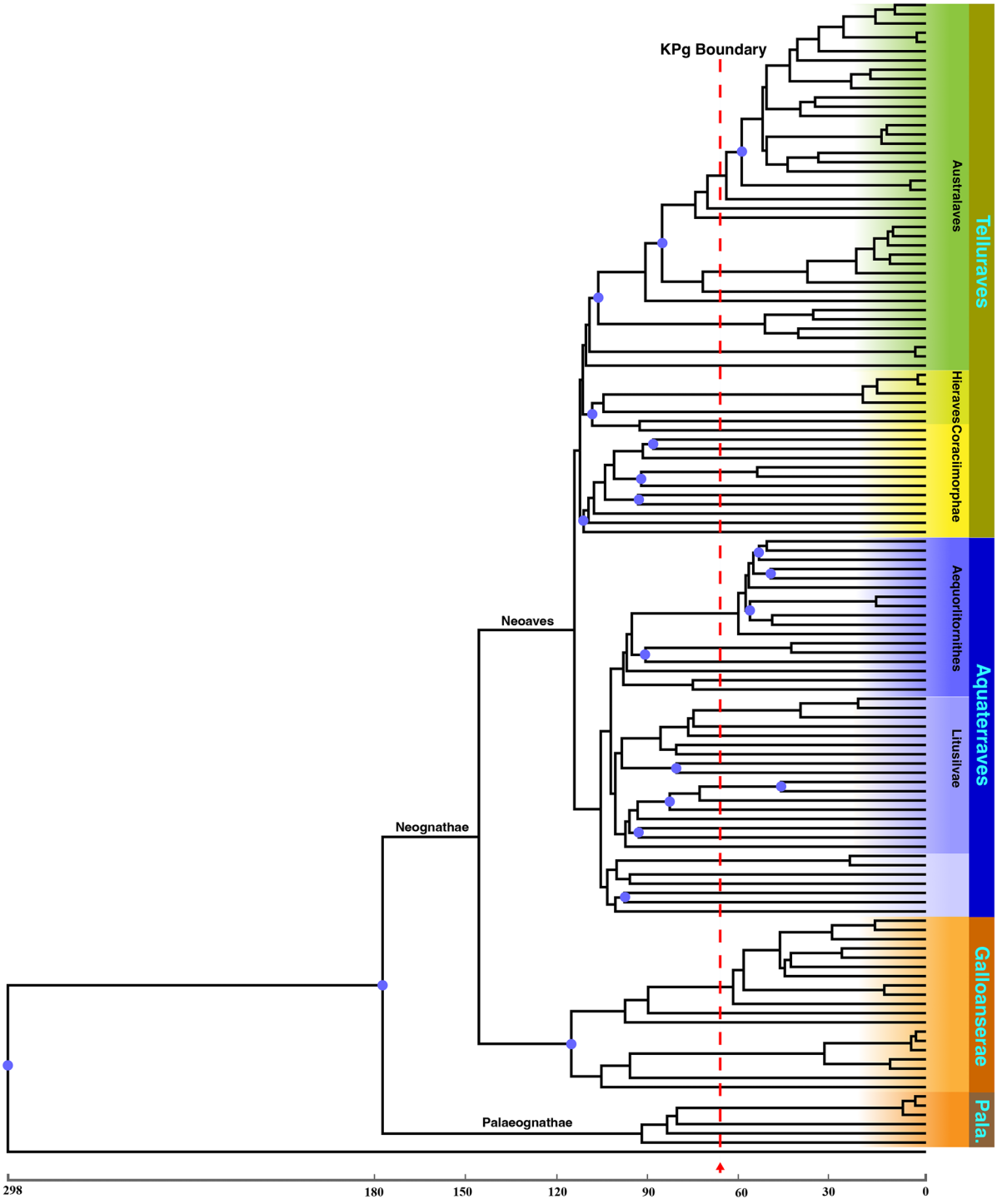

Fig. 1. Dated tree of crown Aves, calibrated using MCMCtree (6) and incorporating 20 fossil constraints, including the eight fossil bounds from table 1 of Claramunt et al. (1). We set the oldest fossil of Ichthyornithes as the minimum bound for crown Aves, following the suggestion by Claramunt et al. (1). Nodes constrained by fossil bounds are marked with violet dots. Species arrangement on the tree aligns with fig. 2 in Wu et al. (2). Note that divergence estimates of early crown Aves are older than those proposed by Wu et al. (2), providing further evidence for a Cretaceous radiation of crown Aves.

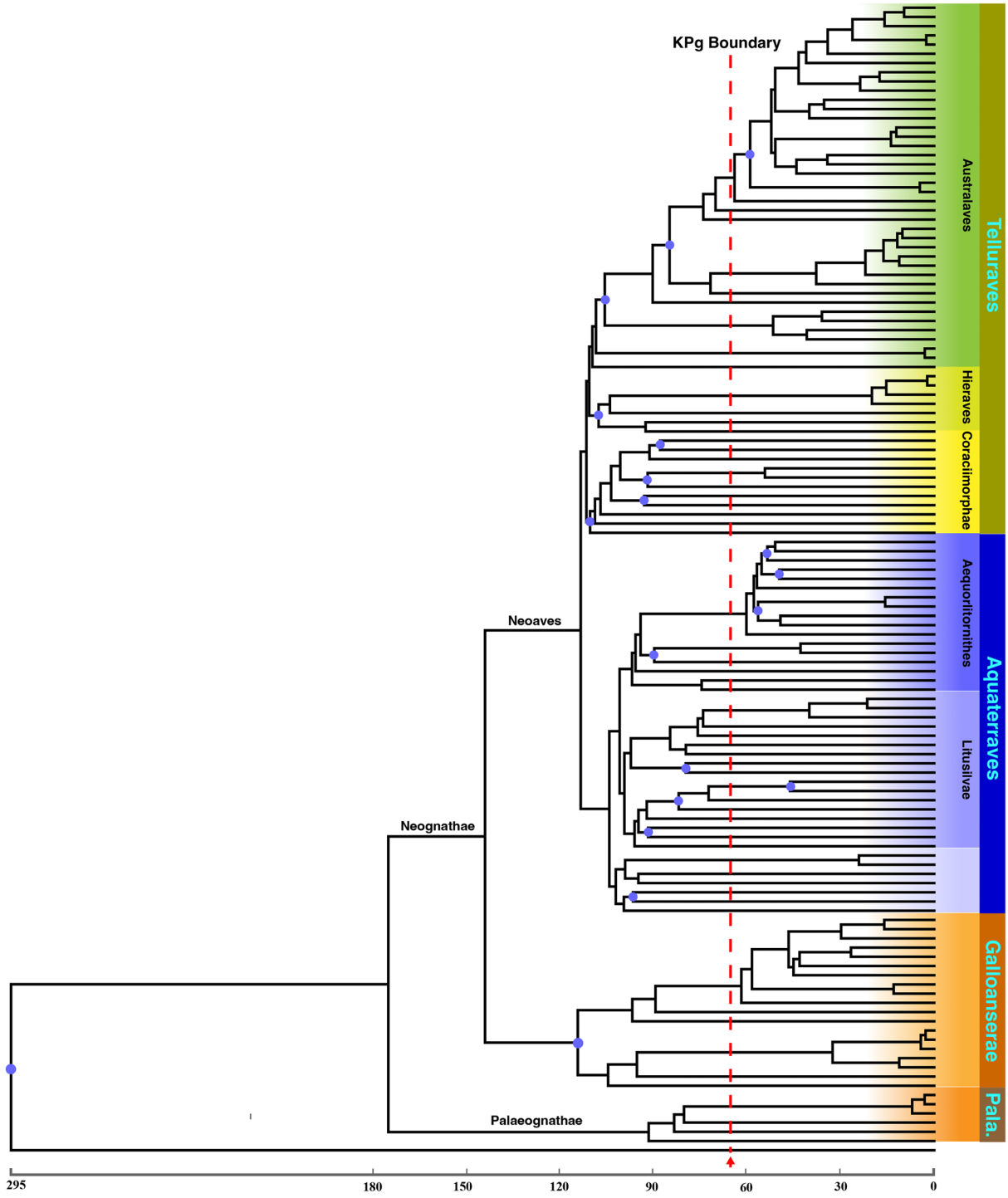

Fig. 2. Dated tree of crown Aves, calibrated using MCMCtree (6) and incorporating 19 fossil constraints. Nodes constrained by fossil bounds are marked with violet dots. Species arrangement on the tree aligns with Fig. 2 in Wu et al. (2). Following Claramunt et al. (1), we relaxed the fossil bound previously imposed on the node between Palaeognathae and Neognathae (crown Aves). Note that divergence estimates of early crown Aves are older than those proposed by Wu et al. (2), providing additional evidence for a Cretaceous radiation of crown Aves. This finding highlights that the dating results of Claramunt et al. (1), which suggest a post-KPg radiation of crown Aves, cannot be replicated using standard clock dating methodology, as noted by Referee 1 commenting on their earlier work (7).

artificially altered fossil bounds. In contrast, our results are robust, grounded in the appropriate application of fossil constraints, and capable of withstanding extensive sensitivity tests.

ACKNOWLEDGMENTS. This work was supported by funds from the National Natural Science Foundation of China (Grant 32020103005 to S.W. and Y.Q.; Grant 31772441 to S.W. and F.E.R.; Grant 42172015 to C.Q.), and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

1. S. Claramunt *et al*., Calibrating the genomic clock of modern birds using fossils. *Proc. Natl. Acad. Sci. U.S.A.* **121**, e2405887121 (2024).
2. S. Wu *et al.*, Genomes, fossils, and the concurrent rise of modern birds and flowering plants in the Late Cretaceous. *Proc. Natl. Acad. Sci. U.S.A.* **121**, e2319696121 (2024).
3. I. Weidig, New birds from the lower Eocene Green River formation, North America. *Rec. Aust. Mus.* **62**, 29–44 (2010).
4. H. Tobin, D. Waugh, J. Clarke, A new zygodactylid species indicates the persistence of stem passerines into the early Oligocene in North America. *BMC Evol. Biol.* **19**, 1–9 (2019).
5. C. Tambussi, F. Degrange, R. De Mendoza, E. Sferco, S. Sergrio, A stem anseriform from the early Palaeocene of Antarctica provides new key evidence in the early evolution of waterfowl. *Zool. J. Linn. Soc.* **186**, 673–700 (2019).
6. M. dos Reis, Z. Yang, Approximate likelihood calculation for Bayesian estimation of divergence times. *Mol. Biol. Evol.* **28**, 2161–2172 (2011).
7. J. Stiller *et al.*, Complexity of avian evolution revealed by family-level genomes. *Nature* **629**, 851–860 (2024), 10.1038/s41586-024-07323-1.
8. D. Field, J. Benito, A. Chen, J. Jagt, D. Ksepka, Late Cretaceous neornithine from Europe illuminates the origins of crown birds. *Nature* **579**, 397–401 (2020).
9. J. Clarke, C. Tambussi, J. Noriega, G. Erickson, R. Ketcham, Definitive fossil evidence for the extant avian radiation in the Cretaceous. *Nature* **433**, 305–308 (2005).
10. E. Roberts *et al.*, New age constraints support a K/Pg boundary interval on Vega Island, Antarctica: Implications for latest Cretaceous vertebrates and paleoenvironments. *GSA Bull.* **135**, 867–885 (2023).
11. N. Brocklehurst, D. Field, Tip dating and Bayes factors provide insight into the divergences of crown bird clades across the end-Cretaceous mass extinction. *Proc. Biol. Sci.* **291**, 20232618 (2024).