the TIMETREE of LIFE

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Crocodylians (Crocodylia)

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Abstract

Crocodylia (23 sp.) includes the living alligators and caimans (Alligatoridae), crocodiles (Crocodylidae), and gharials (Gavialidae). Relatives of Alligatoridae and possibly Gavialidae first appear in the early Campanian of the late Cretaceous (~80 million years ago, Ma), but some molecular estimates place the earliest split within Crocodylia before 150 Ma. Estimating divergences within Crocodylia is complicated by unresolved conflict over how living and extinct gharials are related to alligatorids and crocodylids. If Gavialidae and Crocodylidae are close relatives, their divergence could be anywhere between 20 and 80 Ma.

Crocodylia includes the alligators, caimans, crocodiles, and gharials found throughout the world's tropics (Fig. 1). Twenty-three living species are currently recognized (*1*), though some probably represent cryptic species complexes (*2–4*). The fossil record of the group extends to the early part of the Campanian (84–71 Ma) and includes over 150 known species, with many more awaiting description (*5*). They are semiaquatic ambush predators and include the largest living reptiles. Some of these species are used in the exotic leather industry and, as such, are important economic resources for impoverished nations; others are critically endangered. Crocodylians are central to research in developmental biology, osmoregulation, cardiophysiology, paleoclimatology, sex determination, population genetics, paleobiogeography, functional morphology, and reptile genomics. Their dense fossil record, with first appearance data throughout the clade's stratigraphic range, gives us an excellent opportunity to empirically test methods used to estimate divergence times from molecular data (*6*). In this paper, I discuss divergence times within Crocodylia based both on the fossil record and on the nucleotide sequence data.

Virtually all data agree on the monophyly of Alligatoridae, including the two living alligators (*Alligator*) and six or more living caiman species. Among caimans, the dwarf or smooth-fronted caimans (*Paleosuchus*) are basal to other members of the group. There is consensus that 11 species of crocodile (*Crocodylus*) form a clade, with a 12th—the African Slender-snouted Crocodile (*Mecistops cataphractus*)—being basal to either *Crocodylus* or the African dwarf crocodiles (*Osteolaemus*). The Indonesian False Gharial (*Tomistoma schlegelii*) is universally seen as being closer to crocodiles than to alligators. These groups—Alligatoridae and Crocodylidae—belong to more inclusive groups (Alligatoroidea and Crocodyloidea, respectively) that include extinct relatives of the "families" (*5*).

Relationships among derived caimans and within *Crocodylus* are unclear, but this reflects a lack of resolution in most data sets, probably as a result of the recency of their divergences (*7, 8*). The only real controversy involves the Indian Gharial, *G. gangeticus*. Morphological data strongly support a distant relationship and comparatively ancient divergence (Mesozoic, minimally 80 Ma) between *Gavialis* and other living crocodylians. *Tomistoma*, based on these data, joins *Crocodylus*, *Mecistops*, and *Osteolaemus* within Crocodylidae. Molecular data sets usually support a close relationship between *Gavialis* and *Tomistoma* and a much more recent divergence between them. In this case, *Gavialis* and *Tomistoma* would form a monophyletic Gavialidae and extant Crocodylidae would be limited to *Osteolaemus*, *Mecistops*, and *Crocodylus* (*5, 9–15*).
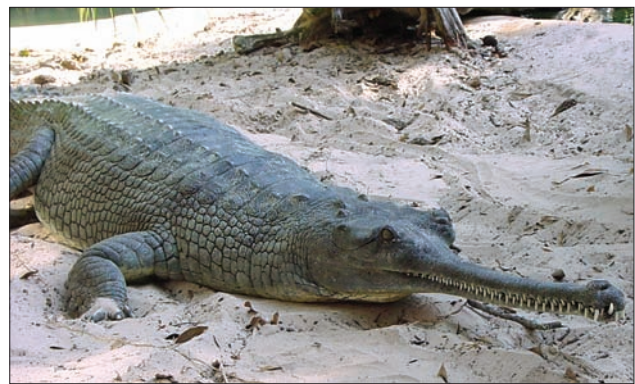
Fig. 1 The Gharial (*Gavialis gangeticus*) from the Indian subcontinent. Credit: C. A. Brochu.
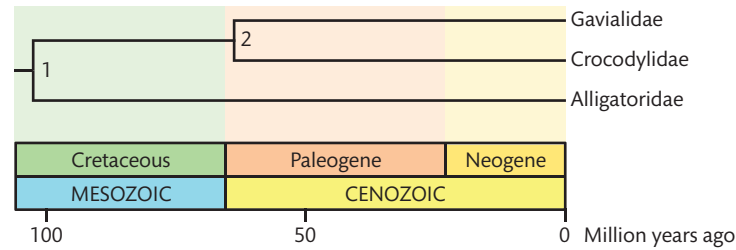
Fig. 2 A timetree of crocodylians (Crocodylia). Divergence times are shown in Table 1.

This complicates efforts to use internal calibration points for the group and compare molecular divergence times with the fossil record. Divergence estimates between *Tomistoma* and *Gavialis* make little sense when their fossil relatives cannot be arranged on the tree. There are what appear to be robust calibration points within the clade, but some of these (e.g., the *Tomistoma–Crocodylus* split) make little sense when the fossils bracketing the divergence point exclude lineages that molecular data argue should be included.

Several first appearances within Crocodylia are robust. The oldest alligatorid, *Navajosuchus mooki*, is from the lowermost Paleocene (66–62 Ma). The lineage including *Alligator* was generally localized in North America and Eurasia at a time when non-marine vertebrate sampling is good. Caimans also first appear in the Paleocene (66–56 Ma), albeit with a spottier fossil record. Minimal morphological divergence between the oldest known alligatorids and their closest extinct relatives suggests that the last common ancestor of alligators and caimans lived at or near the Cretaceous–Paleogene boundary, approximately 66 Ma (*16, 17*). Slightly older calibrations used in some analyses (*15, 18*) are based on arbitrary extensions of the fossil date (*17*) and may be close to the origination time, but this is difficult to test.

The tomistomine–crocodyline split can be placed minimally in the Ypresian stage of the Eocene (56–49 Ma) based on the tomistomine *Kentisuchus* and the crocodyline *Kambara*. The degree of disparity among early crocodylids and close relatives is minimal (*19–21*).

Several Campanian (84–71 Ma) alligatoroids are known (*22–25*), and the oldest crocodyloid is from the Maastrichtian (71–66 Ma) (*5*). Older crocodyloid fossils have been reported (*26*), but these are based on fragmentary material that cannot be reliably assigned to Crocodylia. The basal-most alligatoroids and crocodyloids are morphologically very similar, and the fit between stratigraphic and phylogenetic occurrence is good (*5*). The inference is that their divergence is probably not much earlier than their first fossil appearances. But although the fossil record of late Jurassic (161–146 Ma) and early Cretaceous (146–100 Ma) crocodyliforms is excellent, the record through the middle Cretaceous, especially of close relatives of Crocodylia, is much less complete (*27*). Phylogenetic uncertainty over *Gavialis* and its putative fossil relatives also complicates the situation.

These dates are consistent with molecular clock estimates based on distance data (*28, 29*). *Gavialis* is the only exception—most (though not all) molecular data posit a Cenozoic (66–0 Ma) divergence between them (Table 1, Fig. 2). This differs from most prominent conflicts between molecular and morphological data; in most cases, molecular data suggest substantially older divergences than the fossil record suggests. In this case, molecular estimates are tens of millions of years younger than the earliest known fossils. For this reason, the phylogenetic identity of Cretaceous and Paleocene gavialoids is controversial (*9, 30*).

Application of quartet dating to several mitochondrial genes showed a strong relationship between the ages of the internal calibrations used and the resulting estimate of divergence time between alligatorids and crocodylids. In all cases, two internal calibration points were used: one within Alligatoridae and another within Crocodylidae. Estimates based on post-Eocene calibrations are uniformly younger than those in which one or both calibration points was of Eocene age or older. Estimates based on two calibrations of very different age—one within the past 30 million years and one older than 50 million years—are usually close to the Campanian first appearance datum (*6, 31*).

Recent studies based on mitogenomic data resulted in widely different divergence estimates for the same nodes. The first (*14*) used nonparametric rate smoothing (NPRS), penalized likelihood (PL), and Bayesian

Table 1. Divergence times (Ma) and their confidence/credibility intervals (CI) within Crocodylia.

Timetree		Estimates								
Node	Time	Ref. (*6*)(a)	Ref. (*6*)(b)	Ref. (*6*)(c)	Ref. (*14*)(a)		Ref. (*14*)(b)		Ref. (*15*)(a)	
		Time	Time	Time	Time	CI	Time	CI	Time	CI
1	102.6	41.0	71.0	107.0	137.0	144–130	164.0	184–144	101.0	104–98
2	63.8	–	–	–	74.0	80–68	85.0	101–69	47.0	50–44

Timetree		Estimates (Continued)								
Node	Time	Ref. (*15*)(b)		Ref. (*15*)(c)		Ref. (*16*)(a)		Ref. (*16*)(b)		Ref. (*16*)(c)	
		Time	CI	Time	CI	Time	CI	Time	CI	Time	CI
1	102.6	97.0	102–92	–	106–100	33.0	39–27	42.0	48–36	55.0	76–34
2	63.8	49.0	54–44	49.0	53–45	–	–	–	–	–	–

Timetree		Estimates (Continued)					
Node	Time	Ref. (*16*)(d)		Refs. (*10, 32*) (a)		Refs. (*10, 32*) (b)	
		Time	CI	Time	CI	Time	CI
1	102.6	78.0	99–57	112.0	116–110	147.0	155–142
2	63.8	–	–	–	–	73.0	78–71

Note: Node times in the timetree represent the mean of time estimates from different studies. In ref. (*6*), time estimates were generated from the quartet analysis of four mitochondrial genes with both calibrations from the Neogene (a), with one Neogene and one Paleogene calibration (b), and with calibrations both from the Paleogene (c). In ref. (*14*), NPRS (a) and Bayesian (b) analyses of mitogenomic amino acid sequences were conducted. In ref. (*15*), estimates from PL (a), NPRS (b), and Bayesian (c) analyses on mitogenomic amino acid sequences using an internal calibration are shown; additional analyses using nucleotide sequences or excluding the internal calibration are very similar. In ref. (*16*), PL estimates from nucleotide (a, b) and amino acid (c, d) alignments and based on one (a, c) or five (b, d) calibration points are shown. Refs. (*13, 42*) report NPRS studies of two nuclear and four mitochondrial genes where *Gavialis* was excluded (a) and included (b).

methods. It estimated a gavialid–crocodylid divergence near fossil predictions (albeit with a different topology), and the *Gavialis–Tomistoma* divergence (36–48 Ma) postdates current fossil evidence, but other estimates were substantially older (Table 1), including a late Jurassic (161–146 Ma) alligatorid–crocodylid split.

The second study (*15*) was similar to its predecessor, but with improved taxon sampling and an internal calibration point (alligator–caiman) for some analyses. Recovered dates were much younger than in the preceding mitogenomic analysis, including a *Gavialis–Tomistoma* divergence of 28–22 Ma (Table 1). The alligatorid–crocodylid divergences were slightly older than fossil first appearances (~100 Ma).

These two studies were operationally very similar. They used similar mitogenomic data and dating methods. Outgroup sampling was nearly identical, and they used the same external calibration points. Estimates in the second study (*15*) did not change appreciably

when the alligator–caiman calibration was excluded. The most significant difference appears to be the lower bound of one of the external calibration points (marsupial–eutherian), which was cut from 174 Ma (*14*) to 138 Ma (*15*). This may have inflated the divergence estimates in the earlier study and reinforces the importance of calibration choice in molecular divergence time estimation.

Dates reported from *RAG1* data using PL (*18*) appear anomalous at first. As with the mitogenomic studies, a relationship between estimate and calibration choice was noted; but aligned nucleotides put the alligatorid–crocodylid split between 42 and 33 Ma (Table 1) and the alligator–caiman split between 21 and 17 Ma. Fossil first appearances are two to three times older. But when analyzed as amino acid sequences, 95% confidence intervals around dates estimated for the same divergence points either include, or come close to including, first appearances from fossils (Table 1). This is in contrast to the

second mitogenomic study (*15*), which found no significant difference between estimates from nucleotide and amino acid sequences.

Additional NPRS estimates were obtained for the alligatorid–crocodylid split using a data set (12 taxa, 3667 basepairs) combining nuclear (*RAG1* and *c-mos*) and mitochondrial (*12s*, *16s*, cytochrome *b*, $tRNA^{glu}$ with a flanking portion of *nd6*) genes (*10, 13, 32*) that can be analyzed using the same model (HKY + G + I). The dates were obtained using r8s, version 1.71 (*33*).

Two sets of dates were obtained. The first considered trees in which *Gavialis* was excluded, and only the alligatorid–crocodylid divergence time was estimated. The second was based on trees including *Gavialis* as the closest relative of *Tomistoma*, and two dates—the basal split within Crocodylia and the crocodylid–gavialid split—were estimated. In the first case, four internal constraints were used: *Alligator mississippiensis–Alligator sinensis* (20 Ma), *Alligator*–caiman (64 Ma), *Tomistoma–Crocodylus* (54 Ma), and *Osteolaemus–Crocodylus* (30 Ma). When *Gavialis* was included in the analysis, the *Tomistoma–Crocodylus* calibration was not used. The calibrations were used as upper temporal constraints rather than fixed points.

The resulting estimates for the alligatorid–crocodylid split depend on whether *Gavialis* is included. Trees excluding *Gavialis* put the alligatorid–crocodylid split at ~112 Ma, and trees including it put the same split at ~147 Ma. There are several factors that might explain this disparity, including asymmetry in the distribution of calibrations. Alligatorids often have longer branches and higher rates of evolution than crocodylids, and relative rate tests often reject a single-rate model for Crocodylia (*9, 11, 31*). This is true for the data set analyzed here. Because the *Crocodylus–Tomistoma* calibration point was not used when *Gavialis* was included, the resulting trees relied more heavily on calibrations from among alligatorids.

If we take the more ancient mitogenomic estimates literally, there are surprisingly few stratigraphic implications. The fossil record, read literally, shows no drop in diversity at the Cretaceous–Paleogene boundary (*34, 35*). Molecular divergence estimates would draw multiple extant lineages back to the Cretaceous, indicating increased survivorship across a boundary that, for the group in question, already shows a high level of survivorship. They would, however, diminish the apparent drop in crocodylian diversity during the late Eocene and Oligocene, followed by an increase in diversity during the early Miocene (*27*).

The most important implication comes from younger rather than older molecular dates. Many putative gavialoids and tomistomines predate molecular estimates of their divergence (*15, 29, 36*) by tens of millions of years. We continue to reevaluate these fossils, but for now they continue to support a minimum divergence of 80 Ma between *Gavialis* and *Tomistoma*, even if they are constrained as closest living relatives (*13, 37*).

Extension of basal divergences to the late Jurassic or early Cretaceous brings them within the time frame of Gondwanan breakup. It would be tempting to argue that early divergences lend support to a vicariance model for crocodylian historical biogeography. However, the biogeographic distribution of crocodylians, with or without fossils, does not match a vicariant pattern (*38*). Most extant lineages are fully capable of withstanding exposure to salt water (*39*), and whether one relies on the preferred morphological or molecular tree, branching order is inconsistent with plate tectonic history. Dispersal remains the best explanation for the distribution of most crocodylian clades.

Acknowledgments

References

1. J. P. Ross, *Crocodiles—Status Survey and Conservation Action Plan*, 2nd ed. (International Union for the Conservation of Nature, Gland, Switzerland, 1998).
2. G. Amato, J. Gatesy, in *Molecular Ecology and Evolution: Approaches and Applications*, B. Schierwater, B. Streit, G. P. Wagner, R. DeSalle, Eds. (Birkhäuser Verlag, Basel, 1994), pp. 215–226.
3. D. A. Ray, P. S. White, H. V. Duong, T. Cullen, L. D. Densmore, in *Crocodilian Biology and Evolution*, G. Grigg, F. Seebacher, C. E. Franklin, Eds. (Surrey Beatty and Sons, Sydney, 2001), pp. 58–63.
4. A. Schmitz *et al.*, *C. R. Palevol.* **2**, 703 (2003).
5. C. A. Brochu, *Ann. Rev. Earth and Planet. Sci.* **31**, 357 (2003).
6. C. A. Brochu, *Evolution* **58**, 1375 (2004).
7. C. A. Brochu, L. D. Densmore, in *Crocodilian Biology and Evolution*, G. Grigg, F. Seebacher, C. E. Franklin, Eds. (Surrey Beatty and Sons, Sydney, 2001), pp. 3–8.
8. L. R. McAliley *et al.*, *Mol. Phylogenet. Evol.* **39**, 16 (2006).
9. J. Harshman, C. J. Huddleston, J. P. Bollback, T. J. Parsons, M. J. Braun, *Syst. Biol.* **52**, 386 (2003).

10. R. E. Willis, L. R. McAliley, E. D. Neeley, L. D. Densmore, *Mol. Phylogenet. Evol.* **43**, 787 (2007).

11. C. A. Brochu, *Syst. Biol.* **46**, 479 (1997).

12. S. W. Salisbury, P. M. A. Willis, *Alcheringa* **20**, 179 (1996).

13. J. Gatesy, G. Amato, M. Norell, R. DeSalle, C. Hayashi, *Syst. Biol.* **52**, 403 (2003).

14. A. Janke, A. Gullberg, S. Hughes, R. K. Aggarwal, U. Arnason, *J. Mol. Evol.* **61**, 620 (2005).

15. J. Roos, R. K. Aggarwal, A. Janke, *Mol. Phylogenet. Evol.* **45**, 663 (2007).

16. C. A. Brochu, *J. Vert. Paleo.* **24**, 856 (2004).

17. J. Müller, R. R. Reisz, *BioEssays* **27**, 1069 (2005).

18. A. F. Hugall, R. Foster, M. S. Y. Lee, *Syst. Biol.* **56**, 543 (2007).

19. P. M. A. Willis, R. E. Molnar, J. D. Scanlon, *Kaupia* **3**, 27 (1993).

20. C. A. Brochu, *Palaeontology* **50**, 917 (2007).

21. P. Piras, M. Delfino, L. del Favero, T. Kotsakis, *Acta Pal. Pol.* **52**, 315 (2007).

22. J. E. Martin, *J. Vert. Paleontol.* **27**, 362 (2007).

23. T. E. Williamson, *J. Vert. Paleontol.* **16**, 421 (1996).

24. X.-C. Wu, A. P. Russell, D. B. Brinkman, *Can. J. Earth Sci.* **38**, 1665 (2001).

25. A. K. Martin, C. J. H. Hartnady, *J. Geophys. Res.* **91**, 4767 (1986).

26. M. J. Benton, in *The Fossil Record 2*, M. J. Benton, Ed. (Chapman & Hall, London, 1993), pp. 681–715.

27. P. J. Markwick, *Paleobiology* **24**, 470 (1998).

28. L. D. Densmore, in *Evolutionary Biology*, M. K. Hecht, B. Wallace, G. H. Prance, Eds. (Plenum Press, New York, 1983), Vol. 16, pp. 397–465.

29. C. A. Hass, M. A. Hoffman, L. D. Densmore, L. R. Maxson, *Mol. Phylogenet. Evol.* **1**, 193 (1992).

30. C. A. Brochu, *J. Vert. Paleontol.* **24**, 610 (2004).

31. C. A. Brochu, *J. Paleontol.* **78**, 7 (2004).

32. P. S. White, L. D. Densmore, in *Crocodilian Biology and Evolution*, G. Grigg, F. Seebacher, C. E. Franklin, Eds. (Surrey Beatty and Sons, Sydney, 2001), pp. 29–37.

33. M. J. Sanderson , r8s (Analysis of Rates of Evolution), version 1.71 (University of California at Davis, Davis, CA, 2006).

34. P. J. Markwick, *Geological Society of America Abstracts with Programs*, A (1994).

35. D. Vasse, S. Hua, *Oryctos* **1**, 65 (1998).

36. L. D. Densmore, H. C. Dessauer, *Comp. Biochem. Physiol.* **77B**, 715 (1984).

37. C. A. Brochu, *J. Vert. Paleontol.* **20**, 1 (2000).

38. C. A. Brochu, in *Crocodilian Biology and Evolution*, G. Grigg, F. Seebacher, C. E. Franklin, Eds. (Surrey Beatty and Sons, Sydney, 2001), pp. 9–28.

39. L. E. Taplin, G. C. Grigg, *Amer. Zool.* **29**, 885 (1989).