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the **TIMETREE** *of* **LIFE**

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Turtles (Testudines)

H. Bradley Shaffer

Department of Evolution and Ecology, University of California, Davis, CA 95616, USA (hbshaffer@ucdavis.edu)

Abstract

Living turtles and tortoises consist of two major clades (Cryptodira and Pleurodira), 14 families, and ~313 living species. Time-calibrated phylogenetic analyses can provide basic insights into the tempo of turtle evolution. Molecular phylogenetic analyses have confirmed that most families are demonstrably monophyletic, as are Cryptodira and Pleurodira. A time-calibrated analysis of all living families of turtles, which spans ~210 million years, demonstrates that many of the most-endangered clades are over 100 million years old and are represented by one or a few species, while the most species-rich families tend to be relatively young.

As a group, the turtles, tortoises, terrapins, and marine turtles (collectively, the Order Testudines) are one of the most instantly recognizable and well-known clades of non-avian reptiles (Fig. 1). Whether kept as pets, revered in temples, or slaughtered for the marketplace, turtles and tortoises are an integral and sometimes sacred part of many societies. Recent morphological and molecular phylogenetic studies (the latter are primarily mitochondrial) have led to considerable reshuffling of generic boundaries and taxonomic instability as apparently non-monophyletic groups have been identified and reclassified (1). However, at deeper levels, most researchers agree that the 313 living turtle species are distributed among 14 monophyletic families (2). Although species-level diversity is modest, turtles are found in most major habitats and continents on Earth and many island systems.

Marine species (Cheloniidae and Dermochelyidae) may exceed 2 m in total length, and are found worldwide in temperate and tropical oceans. Tortoises (Testudinidae) and some Emydidae and Geoemydidae are exclusively terrestrial; tortoises in particular have invaded many of the world's deserts and some oceanic islands. Among their many unique biological features, turtles are particularly well known for several unique skeletal features, including the “anapsid” skull condition, the lack of teeth, and the shell. Testudines tend to be

very long-lived, and instances of >100-year-old individuals are known (3). They are also extremely variable in their sex-determination mechanisms, and exhibit both genetic- and temperature-dependent sex determination.

Recently, turtles have emerged as one of the most threatened major clades of vertebrates, with 132 of 201 evaluated species listed in the highest categories of endangerment (Extinct, Extinct in the wild, Critically Endangered, Endangered, Vulnerable) by the IUCN (<http://www.redlist.org/>). In this paper, I review the phylogenetic relationships and molecular divergence times of the families of turtles (Fig. 2).

The monophyly of turtles has never been questioned. It is based on the derived characters associated with a shell of ribs fused to overlying dermal bones inside of which lie the girdles (4). For the last 130 years, the living turtles have been divided into two reciprocally monophyletic clades (5). Pleurodira, or the side-necked turtles, retract their necks into the shell by bending the neck in a horizontal plane (6). Living pleurodires have relatively modest diversity, with three families and ~86 species (2), and are restricted to the southern continents of South America, Africa, and Australia/New Guinea, although fossil taxa were more widely distributed (6). Cryptodira, or the hidden-neck turtles, retract their head by bending the neck in the vertical plane. They are more diverse, with 11 families and about 227 species (2). Cryptodires are distributed across all temperate and tropical regions



Fig. 1 A kinosternid species (*Sternotherus carinatus*) endemic to large rivers in the south-central United States. Photo credit: H. B. Shaffer.

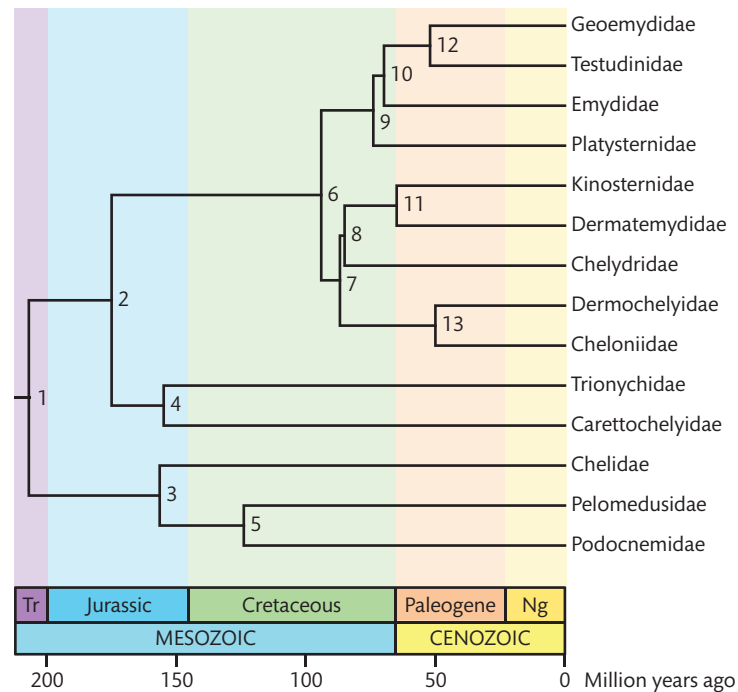


Fig. 2 A timetree of turtles (Testudines). Divergence times are show in Table 1. Abbreviations: Ng (Neogene) and Tr (Triassic).

on Earth, although their primary diversity is restricted to northern continents. Morphological hypotheses of the interrelationships of the living turtles, including characters diagnosing all nodes, are provided in several recent treatments (4, 7–9). Several novel conclusions regarding interfamilial relationships of turtles were suggested by these morphological studies, including the close relationships of mud turtles (Kinosternidae) and softshell turtles (Trionychidae), the unexpected monophyly of the New-World snapping turtles (Chelydridae) and the Asian Big-headed Turtle (Platysternidae), and the close relationship of the Pig-nosed Turtle (Carettochelyidae) and Trionychidae.

Molecular phylogenetic analyses with broad taxonomic coverage across living turtles began about a decade ago (9), and now include studies with both mitochondrial (9, 10) and nuclear (11–13) sequence data. In addition, several comprehensive analyses of within-family relationships (14–17) have helped to further resolve many aspects of relationships among the living turtles. At least three key results have emerged from this body of molecular work. First, the well-sampled families of turtles have been found to be monophyletic, although many of the contained genera have not. The one possible exception is the largely Old-World pond turtle Family Geoemydidae

(sometimes referred to as Bataguridae). Based on morphological evidence, geoemydids were considered to be paraphyletic with respect to the tortoises (Testudinidae), which were hypothesized to be deeply nested within Geoemydidae (4, 17). While molecular evidence has not supported this hypothesis, it is still an open question whether the diverse Old-World geoemydids and *Rhinoclemmys*, the single New-World genus, are monophyletic with respect to testudinids (17). Second, the relationships of the monotypic Big-headed Turtle Family Platysternidae and the New-World snapping turtles (Family Chelydridae) are finally becoming clear. Based largely on morphological evidence and limited mtDNA data, the two were considered to be closest relatives (4, 9). However, whole mitochondrial genome data (10), *RAG1* nuclear data (12), and combined *RAG1* and mtDNA data (12) indicate that Platysternidae is most closely related to Testudinoidea (Emydidae, Geoemydidae, Testudinidae). The placement of Chelydridae remains uncertain—nuclear and mtDNA suggest affinities with the Kinosternidae and their relatives (12), whereas whole mitochondrial data place them as the closest relative of a clade containing Testudinoidea, the marine turtles, and Platysternidae (10). Third, the phylogenetic placement of the softshell turtles (Trionychidae) remains enigmatic.

Table 1. Divergence times (Ma) and their confidence/credibility intervals (CI) among turtles (Testudines).

Node	Timetree		Estimates			
	Time	Ref. (19)		Ref. (22)		
		Time	CI	Time	CI	
1	207.0	-	-	207	221-193	
2	175.0	175	186-164	-	-	
3	156.5	176	184-168	137	159-115	
4	155.0	155	166-144	-	-	
5	124.0	124	135-113	-	-	
6	94.0	94	100-88	-	-	
7	87.0	87	95-79	-	-	
8	85.0	85	91-79	-	-	
9	74.0	74	93-55	-	-	
10	70.0	70	76-64	-	-	
11	65.0	65	-	-	-	
12	52.0	52	-	-	-	
13	50.0	50	57-43	-	-	

Note: The node times in the timetree are average of times from difference studies. The times for Nodes 11 and 12 are based on fossil dates supported by a cross-validation procedure with molecular time estimates.

Molecular data suggest that softshells and their close relative, the Pig-nosed Turtle (Carettochelyidae), are either closest to the remaining Cryptodira (12) or perhaps represent the first split among all living turtles and fall outside of Cryptodira [suggested as an unlikely possibility in (12) and (22)]. In either case, they are not closely related to the mud turtle clade as suggested based on morphological analyses (10–12).

Because they have a superb fossil record, turtles have long been recognized as excellent candidates for fossil-calibrated molecular phylogenies. In addition, an intriguing early suggestion, based on within-species phylogeographic estimates, that turtles may be characterized by a slow rate of molecular evolution (18) raised the possibility that molecular clocks may be applicable to very ancient phylogenetic events in turtle history. However, only two papers (9, 19) have used fossils to explore divergence times among all of the major lineages of turtles. In the first (9), the primary emphasis was on the impact of including fossils on phylogeny estimation, rather than time estimation. However, one major conclusion from that work was that the major lineages of cryptodiran turtles all appeared to diverge roughly 100 million years ago (Ma), based on evidence from their earliest fossil representatives.

Near *et al.* (19) provided estimates of divergence times for all major clades of living Testudines, as well as a new cross-validation method for identifying potential outlier fossils that may be providing inaccurate divergence dates. Using 10 consistent fossil calibration dates and 4691 basepairs of nuclear (*RAG 1* exon, *R35* intron) and mitochondrial (*cyt b*) data scored for 23 turtles that include representatives of all living families and subfamilies of turtles (12), Near *et al.* provided several new insights into the tempo of turtle evolution (Fig. 2, Table 1). Primary among them are that most of the among-family divergences of turtles are ancient, and predate the Cretaceous–Cenozoic boundary (>66 Ma). However, divergences among the most diverse families of living Cryptodira (Testudinidae, 55 species; Emydidae, 48 species; Geoemydidae, 66 species) are much more recent, with most of the within-family diversification occurring within the last 50 million years. Some aspects of this work have recently been criticized (20), including the antiquity of the Cryptodira–Pleurodira split (8, 23), although molecular analyses of the *RAG-1* gene across tetrapods provides independent evidence in support of the 210 million year age of this event (22). Thus, the fundamental conclusions of Near *et al.* (21) appear to remain valid. In particular, several of the most critically endangered families of Testudines have origins in the Jurassic (200–146 Ma) or Cretaceous (146–66 Ma) and are currently represented by one or a few living species. These lineages contain a great deal of unique phylogenetic history, and are important candidates for conservation action.

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