What Is the True "Papa" Turtle? A Discussion About the Origin of The Clade Testudines from Both Morphological and Molecular Perspectives

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Abstract. Turtles, from the order Testudines has a debatable origin. The oldest defined species of the order was *Proganochelys quenstedti* from the late Triassic. However, the origin or the ancestor of this species has been a hot topic among scientists. This paper discussed the potential origin of the turtles using both the morphological and molecular perspectives. Based on the morphological evidence for the evolution of the defining features, one theory suggested that this order originated from the *Eunotosaurus* africanus which the dorsal ribs fused together first; other theories suggested that it originated from *Odontocheyls semi Testacea* which the ventral ribs fused first. The skull structure is another debatable topic as evolutionary evidence which the current turtles are classified as anapsid due to its lack of temporal *fenestration.* However, many of the potential ancestors showed defining two fenestrations of diapsids or at least some features indicating it was evolving into anapsid. The molecular evidence of mitochondrion cDNA showed that the Testudines clade diverged from the other *Archosauri* in Jurassic which completely overthrow all the hypothesis. Although morphological based theory has its flaw, it was a more convincing theory than the molecular based theory. As some studies suggested that the *Odontotomy's semi Testacea* could lost its dorsal shell inherited from its ancestor, the hypothesized evolutionary route is *E. africanus →Pappocheyl. rosinae →O. semitestacea→P. quenstedti*.

Keywords: *Testudines*, Paleobiology, Evolution, Fossil, *Pappochelys rosinae, Odontocheyls semi Testacea, Proganochelys quenstedti, Eunotosaurus africanus,* Molecular clock, diapsid, anapsid, turtles, reptiles, Archosauria.

1. Introduction

Species from the order Testudines, commonly known as turtles or tortoises, known for its long-life span, is also the oldest existing clade of reptile based on Hox gene evidence [1]. Discovered by German scientists Rainer Schoch and his college in 2015, Pappochelys was thought to be the "grandfather" of turtles as the name suggested [2]. However, the true "papa" of this ancient species remains a debate among scientists. Through different perspectives of molecular and morphological evidence, the blog will examine and discuss the potential candidate for the true ancestor of the turtle.

As new fossil evidence emerges every few years, the candidate for the ancestor of the turtle changes frequently, especially when considering molecular evidence. Before the discovery of molecular evidence which make the true ancestor of the turtle remain a mystery again, there was a logical hypothesis of the evolution of the ancestor of the turtle mostly based on the morphological evidence provided by fossils. The blog will introduce the morphologically hypothesized evolutionary route of turtle in the temporal order.

2. The Potential Ancestors of turtle

2.1. The Oldest Turtle-like Creature

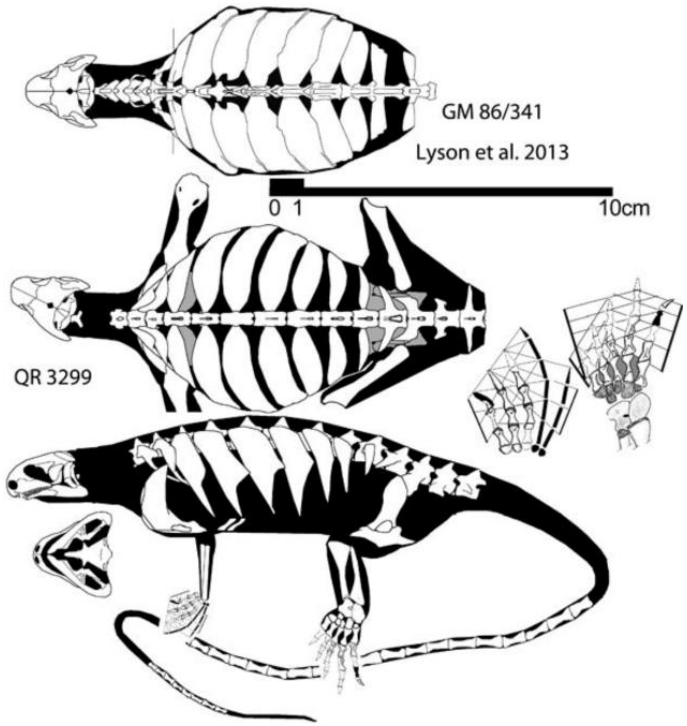

Figure 1. the skeleton of *Eunotosaurus africanus* [3]

The oldest known species which at least morphologically resembles a turtle is *Eunotosaurus africanus*, an extinct species of reptile in the mid to late Permian period that shared multiple characteristics with modern turtles [4].

As shown in figure 1, the reptile has a broadened and thickened dorsal nerve table with widened ventral ribs that have yet to have no ossification to fuse them together. However, it is no doubt that the skeleton of the reptile does resemble the shape of a turtle intuitively to some extent. The species has teeth instead of beak-like modern turtles. The details of the reconstruction on the limb of the creature show that it had 5 fingers which match the number of fingers seen in modern-day terrestrial and semi-aquatic turtles. The fingers curve towards the posterior of the body, indicating they are potentially for digging. The broad dorsal rib which is wider than modern turtles help stabilize the weight center and condenses the muscle for digging [5].

2.2. "Papa" Turtle

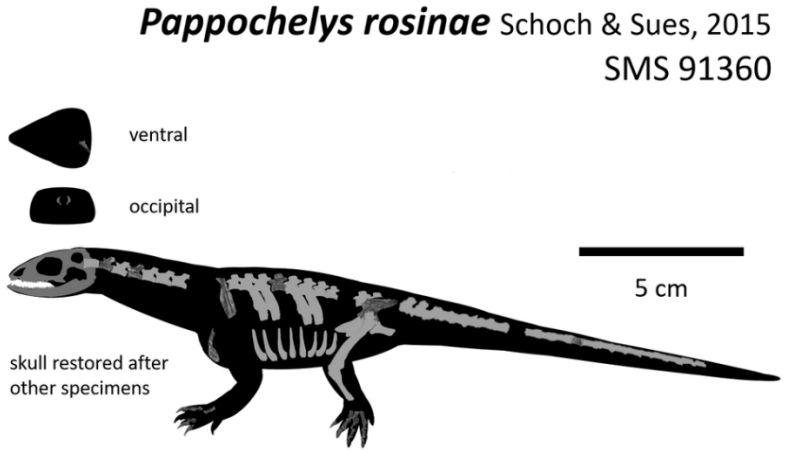

Figure 2. the reconstruction of *Pappocheyls rosinae* based on fossil evidence provided by Schoch & Sues [6].

Pappochelys rosinae is thought to be living in the early Triassic based on fossil evidence around 240 million years ago. As demonstrated by figure 2, P. rosinae shows broadened dorsal ribs and more densely packed ventral ribs which have the potential to evolve into ventral shells resembling the one possessed by *testudines*. The species possess teeth instead of a beak. Fingers curving towards the posterior side are seen in *P. rosinae* as well. The overall body plan is more streamlined while the tailbone is thicker compared to *E. africanus*, suggesting its potential semi-aquatic lifestyle [2].

2.3. Turtle-like Animal with Half Shell and Teeth

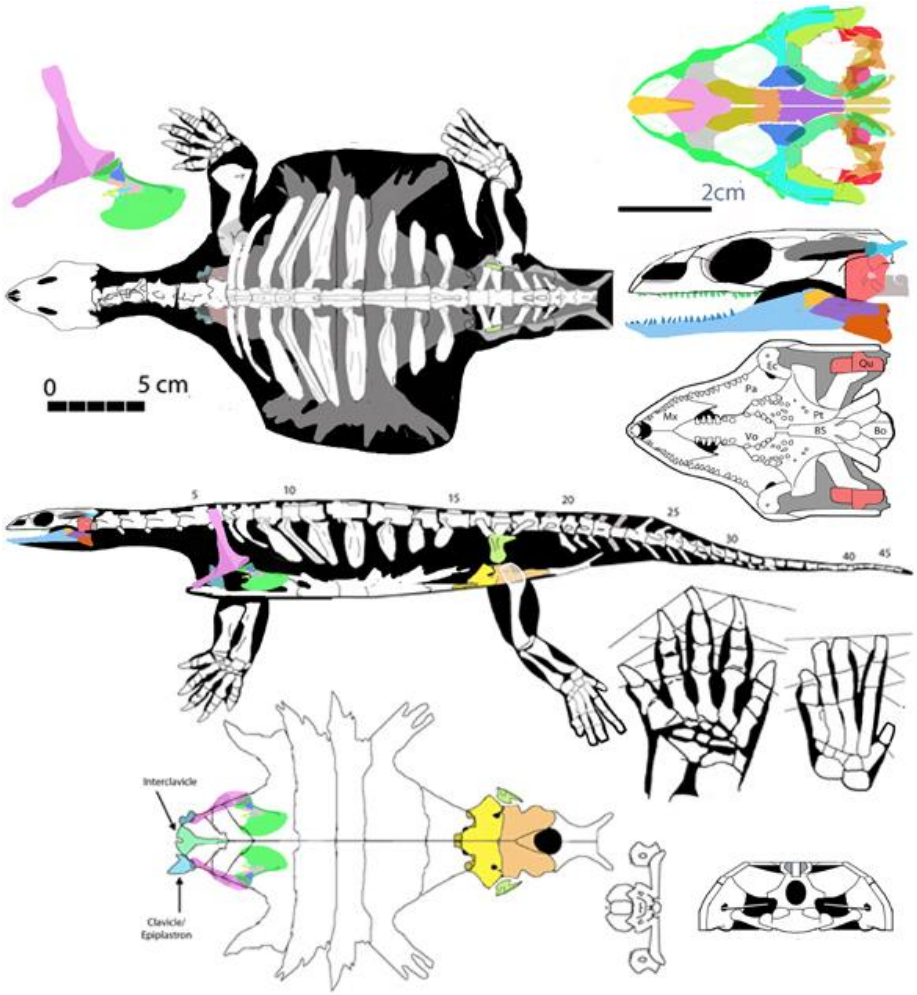

Figure 3. the reconstruction of *Odontocheyls semitestacea* [7].

O. semi Testacea gets its name from its characteristic of having teeth and half of the shell for a modern turtle. The *O. semi Testacea* was living in 232-221.5 MYA in late Triassic. The tailbone is broader than *P. rosinae*. With a streamlined body shape, the chance of *O. semi Testacea* being aquatic or semi-aquatic is high. The ventral ribs of *O. semi Testacea* have completely fused together with clear evidence of ossification between the ventral ribs. The toes on the hind limbs have lost their sharp end and potentially have flipper-like structures between toes to support swimming [7].

2.4. The First Turtle

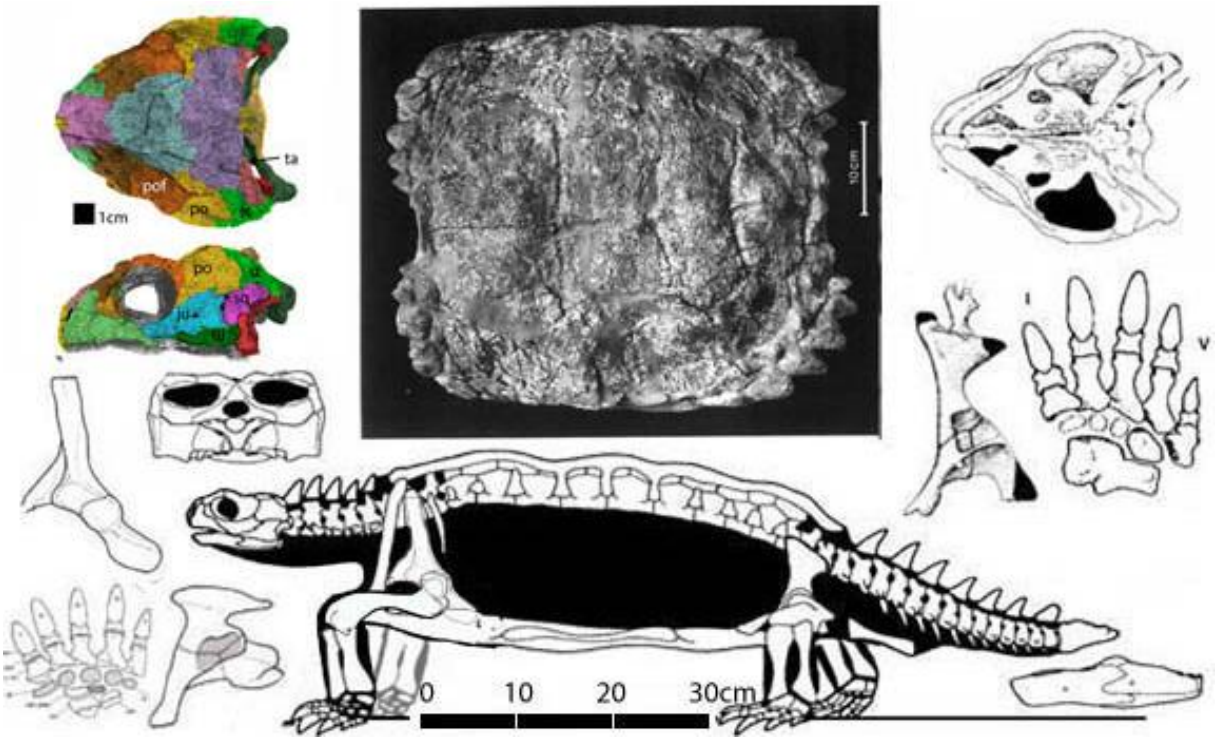

Figure 4. the skeletal structure of *Proganochelys quenstedti* based on fossil evidence [8].

P. quenstedti is the oldest identified species of the order *testudines* found in the late Triassic of 203 million years ago. As shown in figure 4, it possesses characteristics that can be found in all surviving species of turtle such as shell merged from rib form both ventral and dorsal side. This species has a beak and no teeth. However, the relatively oval body shape compare to *O. semitestace* suggests it could be a terrestrial animal. This is further justified by the relatively straight finger which may not be effective in digging or swimming. 3D models of its living environment were constructed based on the geological evidence also justifies that it might be completely terrestrial [9].

3. Discussion of the Morphological Based Evolutionary Route:

A turtle is a tetrapod reptile with shells on both the ventral and dorsal sides formed by rib ossification. Although could be carnivores or herbivores, swimming or digging, having a beak is one of the defining characteristics of the order *testudines* [10]. However, teeth are found in all precursor species of *P. quentesdti* and no beaks are found. There are arguments that beaks were the product of later evolution and species such as *O. semitestace* can still consider to be ancestor species of order *testudines* due to other defining characteristics such as the presence of a shell formed from the rib. Beaks are easier to crack open the hard shells of crustaceans and mollusks compare to teeth which could be one of the main sources of food for semi-aquatic turtle-like species. The beak could be the innovation of *P. quenstedti*, which could be completely terrestrial. Broadened teeth or beak is more effective in chewing plant matter than sharp teeth found in older turtle-like species as shown in fgiure2&3. One of the most convincing pieces of evidence supporting the evolution route of *E. africanus*→*P. rosinae* →*O. semitestace*→*P. quentesdti* is the skull of those species.

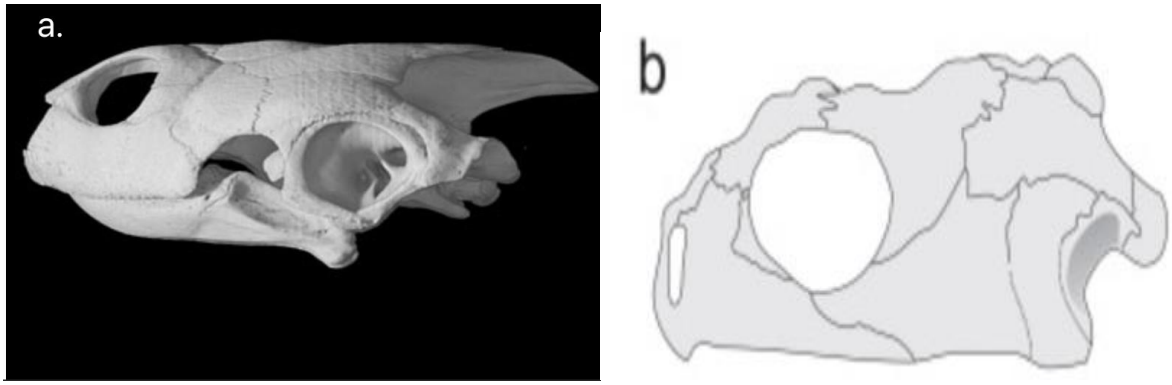

Figure 5. the comparison between the skull of *Podocnemis unifilis* and *Proganochelys quenstedti* a. the skull of *Podocnemis unifilis* [11]. b. The skull of *P. quenstedti* [12].

Anapsid animal is animals that have no fenestration on the temporal face of the skull. Figure 5 demonstrated the comparison between the skull of the modern turtle P.unifilis and the oldest known turtle *P. quenstedti*. They both have no fenestration on the temporal side of skull; thus, they are all anapsid. However, as shown in figure 2, there is a clear fenestration on the temporal side of the skull of *P. rosinae*, indicating its diapsid origin [13]. Similar fenestration can also be found in *O. semitestacea* as shown in figure 3. Although there is no exposed fenestration found on the skull of *E. africanus*, based on a recent study using high-resolution computer tomography, the bone covering the supertemporal face of the skull of juvenile *E. Africanus* indicates it could also be diapsid [14]. Such discoveries seemingly deny the evolutionary route for testudines which the *P. quenstedti* may evolved from different ancestors that are anapsid. Surprisingly, the molecular evidence using GEN bank back up the hypothesis. For modern turtles, analysis of molecular evidence from the cDNA sequences of A, B-lactate dehydrogenase (LDH) as well as a-enolase in mitochondrion using the techniques of neighbor-joining and maximum likelihood show that clade *testudines* diverged from the diapsid reptilian clade *Archosauria* [15]. Using the fenestration on skull as key features in identification is criticized by Rieppel who suggests that turtle is secondarily evolved anapsid from diapsid based on muscle reconstruction [17].

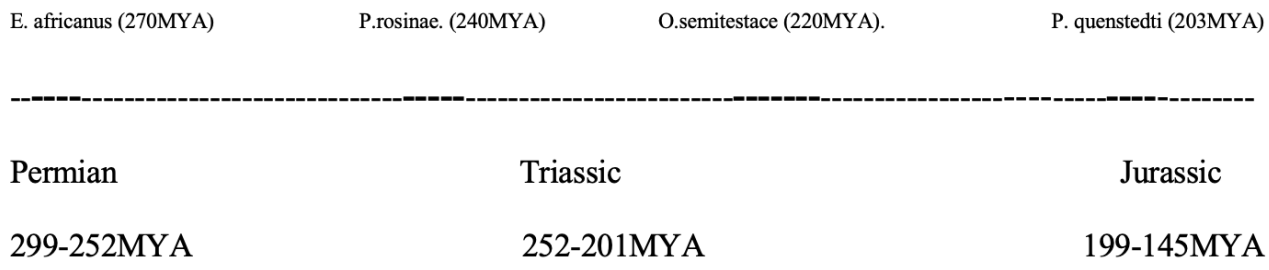

Figure 6. the hypothesized evolutionary timeline for turtle

If the hypothesized evolutionary route as shown by figure 6 is justified, the story of the turtle would begin in the Permian period. A burrowing, potentially semi-aquatic reptile develops wider ribs to lower its weight center to improve its efficiency. Thousands of years later, the offspring of it lived by the water, and found out that by ossification of its broadened ribs, a protective shell can be formed. The shell formed first from the ventral side of the animal potentially to prevent attack from below from *Ichthyosaur* who has lost their pterygoid teeth and should find it difficult to break the shell of the "ancestor" of turtle [17]. Since the shell on one side of the body is already making the body stiff, encasing the whole trunk in shells from both sides may not cause more harm. Potentially due to the feeding habit of benthic mollusks and crustaceans [18], which those animals were able to catch with their low flexibility, the teeth were replaced by beaks. Although the hypnotized evolutionary route for turtles seems well-defined and logical after being aided by evidence from molecular analysis, the molecular analysis also suggests a major error in this theory which is the time.

4. Conflicts of the Evolutionary Timeline as Suggested By Molecular Clock

Molecular clock is a method of estimating the time when two species diverged based on the difference in mitochondrial DNA bases for the same family of genes. After the genes of the two species are aligned, the specific differences are compared. From the number of different and potentially the loci where the bases are different, using the equation of $r = \frac{d(number\ of\ different\ sequences\ in\ pairs)}{T\ (average\ pairwise\ sequence\ divergence)}$) [19].

The staggering result indicates that the clade testudines diverged from the Archosauria clade which is the clade of all modern-day crocs about 151 million years ago [15]. By accepting this evidence, all "turtle-like" ancient animals before the Jurassic could not possibly be the ancestor of modern turtle. As unbelievable as it seems, even *P. quenstedti*, the oldest identified species of testudines found in Triassic as recognized by many scientists, could not be the ancestor of turtle. Fortunately, the more recent molecular clock examined in 2007 has provided a more logical prediction using recombination-activating gene. Indicating that the turtle and croc clade diverged around 210 million years ago in the late Triassic around the time when *P. quenstedti* was discovered. However, since *P. quenstedti* already acquired all characteristics of being a turtle in modern definition [20]. What the first turtle evolved from, is not yet answered

5. Comments and Future Perspectives

It is still a debate about what is the true ancestor of turtles. It seems more logical to believe to a certain extent the correctness of the evolutionary route initiated from *E. africanus*. For molecular evidence, since the rate of mutation in all genes cannot be constant and nevertheless predictable with absolute accuracy, there could be potential deviation enlarged during the calculation which resulted in such contradictory divergent time. While the morphological evidence shows controversial evidence such as the ossification of *P. rosinae* and *E. Africanus* which start from the dorsal side instead of the ventral [2] [3]. The debate goes on if considering all controversial evidence of turtle evolution which there are also scientists suggest that *O. semitestacea* could lose its dorsal shell during evolution [5].

This topic requires further investigation. Since the Triassic period was a period when vertebrate diversification was relatively fast due to the presence of the warm shallow ocean and empty niches was waiting to be filled due to the Permian extinction event [21], new fossil evidence may emerge in the near future, backing up the existing theories or completely denning them and suggest new ones. Turtles were often thought of as the symbol of patients and long living in many cultures, the definition of the ancestor of turtles requires scientists to investigate with the same character as the symbol represents.

References

[1] Böhmer C, Werneburg I. Deep time perspective on turtle neck evolution: chasing the Hox code by vertebral morphology. Sci Rep. 2017 Aug 21; 7 (1): 8939.

[2] Schoch RR, Sues HD. A Middle Triassic stem-turtle and the evolution of the turtle body plan. Nature. 2015 Jul 30; 523 (7562): 584 - 7.

[3] Lyson TR, Bever GS, Bhullar BA, Joyce WG, Gauthier JA. Transitional fossils and the origin of turtles. Biol Lett. 2010 Dec 23; 6 (6): 830 - 3.

[4] Steele RE, David CN, Technau U. A genomic view of 500 million years of cnidarian evolution. Trends Genet. 2011 Jan; 27 (1): 7 - 13.

[5] Joyce WG. The origin of turtles: a paleontological perspective. J Exp Zool B Mol Dev Evol. 2015 May; 324 (3): 181 - 93.

[6] McDonald AT, Wolfe DG, Dooley AC Jr. A new tyrannosaurid (Dinosauria: Theropoda) from the Upper Cretaceous Menefee Formation of New Mexico. PeerJ. 2018 Oct 9; 6: e5749.

[7] Li C, Wu XC, Rieppel O, Wang LT, Zhao LJ. An ancestral turtle from the Late Triassic of southwestern China. Nature. 2008 Nov 27; 456 (7221): 497 - 501.

[8] Burch SH. Complete forelimb myology of the basal theropod dinosaur Tawa hallae based on a novel robust muscle reconstruction method. J Anat. 2014 Sep; 225 (3): 271 - 97.

[9] Dziomber L, Joyce WG, Foth C. The ecomorphology of the shell of extant turtles and its applications for fossil turtles. PeerJ. 2020 Dec 22; 8: e10490.

[10] Alibardi L. Development, structure, and protein composition of the corneous beak in turtles. Anat Rec (Hoboken). 2021 Dec; 304 (12): 2703 - 2725.

[11] Gaffney, Digimorph - Podocnemis Unifilis (Yellow-Spotted Amazon River Turtle). Digimorph. org, 2022.

[12] Schoch RR, Klein N, Scheyer TM, Sues HD. Microanatomy of the stem-turtle Pappochelys rosinae indicates a predominantly fossorial mode of life and clarifies early steps in the evolution of the shell. Sci Rep. 2019 Jul 18; 9 (1): 10430.

[13] Schoch RR, Sues HD. The diapsid origin of turtles. Zoology (Jena). 2016 Jun; 119 (3): 159 - 161.

[14] Bever GS, Lyson TR, Field DJ, Bhullar BA. Evolutionary origin of the turtle skull. Nature. 2015 Sep 10; 525 (7568): 239 - 42.

[15] Mannen H, Li SS. Molecular evidence for a clade of turtles. Mol Phylogenet Evol. 1999 Oct; 13 (1): 144 - 8.

[16] Schoch RR, Sues HD. A Middle Triassic stem-turtle and the evolution of the turtle body plan. Nature. 2015 Jul 30; 523 (7562): 584 - 7.

[17] Lomax DR, Massare JA. Rediscovery of two casts of the historically important 'Proteo-saurus', the first complete ichthyosaur skeleton. R Soc Open Sci. 2022 Nov 2; 9 (11): 220966.

[18] Reisz RR, Head JJ. Palaeontology: Turtle origins out to sea. Nature. 2008 Nov 27; 456 (7221): 450 - 1.

[19] Kumar S, Hedges SB. A molecular timescale for vertebrate evolution. Nature. 1998 Apr 30; 392 (6679): 917 - 20.

[20] alibration Choice, Rate Smoothing, and the Pattern of Tetrapod Diversification according to the Long Nuclear Gene RAG - 1.

[21] Beznosov PA, Clack JA, Lukševičs E, Ruta M, Ahlberg PE. Morphology of the earliest reconstructable tetrapod Parmastega aelidae. Nature. 2019 Oct; 574 (7779): 527 - 531.